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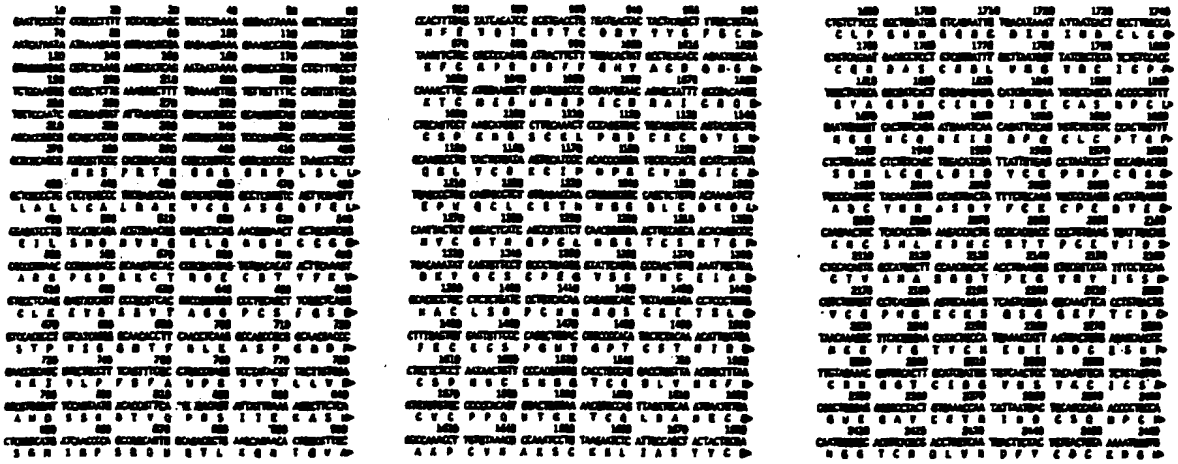

(57) Abstract

The present invention relates to nucleotide sequences of vertebrate *Serrate* genes, and amino acid sequences of their encoded proteins, as well as derivatives (e.g., fragments) and analogs thereof. In a specific embodiment, the Serrate protein is a human protein. The invention further relates to fragments (and derivatives and analogs thereof) of a vertebrate Serrate which comprise one or more domains of the Serrate protein, including but not limited to the intracellular domain, extracellular domain, DSL domain, cysteine rich domain, transmembrane region, membrane-associated region, or one or more EGF-like repeats of a Serrate protein, or any combination of the foregoing. Antibodies to vertebrate Serrate, its derivatives and analogs, are additionally provided. Methods of production of the vertebrate Serrate proteins, derivatives and analogs, e.g., by recombinant means, are also provided. Therapeutic and diagnostic methods and pharmaceutical compositions are provided.In specific examples, isolated Serrate genes, from chick, mouse, *Xenopus* and human, are provided.

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NUCLEOTIDE AND PROTEIN SEQUENCES OF VERTEBRATE SERRATE GENES AND METHODS BASED THEREON

This invention was made in part with government support under Grant numbers GM 29093 and NS 26084 awarded by the Department of Health and Human Services. The government has certain rights in the invention.

1. INTRODUCTION

The present invention relates to vertebrate *Serrate* genes and their encoded protein products, as well as derivatives and analogs thereof. Production of vertebrate Serrate proteins, derivatives, and antibodies is also provided. The invention further relates to therapeutic compositions and methods of diagnosis and therapy.

2. BACKGROUND OF THE INVENTION

Genetic analyses in Drosophila have been extrem ly useful in dissecting the complexity of developmental pathways and identifying interacting loci. However, understanding the precise nature of the processes that underlie genetic interactions requires a knowledge of the protein products of the genes in question.

Embryological, genetic and molecular evidence indicates that the early steps of ectodermal differentiation in *Drosophila* depend on cell interactions (Doe and Goodman, 1985, Dev. Biol. 111:206-219; Technau and Campos-Ortega, 1986, Dev. Biol. 195:445-454; Vässin et al., 1985, J. Neurogenet. 2:291-308; de la Concha et al., 1988, Genetics 118:499-508; Xu et al., 1990, Genes Dev. 4:464-475; Artavanis-Tsakonas, 1988, Trends Genet. 4:95-100). Mutational analyses reveal a small group of zygotically-acting genes, the so called neurogenic loci, which affect the choice of ectodermal cells between epidermal and neural pathways (Poulson, 1937, Proc. Natl. Acad. Sci. 23:133-137; Lehmann t al., 1983, Wilhelm R ux's Arch. Dev. Biol. 192:62-74; Jürgens t al., 1984, Wilhelm Roux's Arch. Dev. Biol.

193:283-295; Wieschaus et al., 1984, Wilhelm Roux's Arch. Dev. Biol. 193:296-307; Nüsslein-Volhard et al., 1984, Wilh lm Roux's Arch. Dev. Biol. 193:267-282). Null mutations in any one of the zygotic neurogenic loci -- *Notch* (*N*), *D lta* (*Dl*), *mastermind* (*mam*), *Enhancer of Split* (*E(spl)*, *neuralized* (*neu*), and *big brain* (*bib*) -- result in hypertrophy of the nervous system at the expense of ventral and lateral epidermal structures. This effect is due to the misrouting of epidermal precursor cells into a neuronal pathway, and implies that neurogenic gene function is necessary to divert cells within the neurogenic region from a neuronal fate to an epithelial fate. *Serrate* has been identified as a genetic unit capable of interacting with the *Notch* locus (Xu et al., 1990, Genes Dev. 4:464-475). These genetic and developmental observations have led to the hypothesis that the protein products of the neurogenic loci function as components of a cellular interaction mechanism necessary for proper epidermal development (Artavanis-Tsakonas, S., 1988, Trends Genet. 4:95-100).

Mutational analyses also reveal that the action of the neurogenic genes is pleiotropic and is not limited solely to embryogenesis. For example, ommatidial, bristle and wing formation, which are known also to depend upon cell interactions, are affected by neurogenic mutations (Morgan et al., 1925, Bibliogr. Genet. 2:1-226; Welshons, 1956, Dros. Inf. Serv. 30:157-158; Preiss et al., 1988, EMBO J. 7:3917-3927; Shellenbarger and Mohler, 1978, Dev. Biol. 62:432-446; Technau and Campos-Ortega, 1986, Wilhelm Roux's Dev. Biol. 195:445-454; Tomlison and Ready, 1987, Dev. Biol. 120:366-376; Cagan and Ready, 1989, Genes Dev. 3:1099-1112).

Sequence analyses (Wharton et al., 1985, Cell 43:567-581; Kidd and Young, 1986, Mol. Cell. Biol. 6:3094-3108; Vässin, et al., 1987, EMBO J. 6:3431-3440; Kopczynski, et al., 1988, Genes Dev. 2:1723-1735) have shown that two of the neurogenic loci, *Notch* and *Delta*, appear to encode transmembrane proteins that span the membrane a single time. The *Notch* gene encodes a ~300 kd protein (we use "Notch" to

denote this protein) with a large N-terminal extracellular domain that includes 36 epidermal growth factor (EGF)-like tandem repeats followed by three other cysteine-rich repeats, designated *Notch/lin*-12 repeats (Wharton, et al., 1985, Cell 43:567-581; Kidd and Young, 1986, Mol. Cell. Biol. 6:3094-3108; Yochem, et al., 1988, Nature 335:547-550). *Delta* encodes a ~100 kd protein (we use "Delta" to denote DLZM, the protein product of the predominant zygotic and maternal transcripts; Kopczynski, et al., 1988, Genes Dev. 2:1723-1735) that has nine EGF-like repeats within its extracellular domain (Vässin, et al., 1987, EMBO J. 6:3431-3440; Kopczynski, et al., 1988, Genes Dev. 2:1723-1735). Molecular studies have lead to the suggestion that Notch and Delta constitute biochemically interacting elements of a cell communication mechanism involved in early developmental decisions (Fehon et al., 1990, Cell 61:523-534).

The EGF-like motif has been found in a variety of proteins, including those involved in the blood clotting cascade (Furie and Furie, 1988, Cell 53: 505-518). In particular, this motif has been found in extracellular proteins such as the blood clotting factors IX and X (Rees et al., 1988, EMBO J. 7:2053-2061; Furie and Furie, 1988, Cell 53: 505-518), in other *Drosophila* genes (Knust et al., 1987 EMBO J. 761-766; Rothberg et al., 1988, Cell 55:1047-1059), and in some cell-surface receptor proteins, such as thrombomodulin (Suzuki et al., 1987, EMBO J. 6:1891-1897) and LDL receptor (Sudhof et al., 1985, Science 228:815-822). A protein binding site has been mapped to the EGF repeat domain in thrombomodulin and urokinase (Kurosawa et al., 1988, J. Biol. Chem 263:5993-5996; Appella et al., 1987, J. Biol. Chem. 262:4437-4440). The *Drosophila Serrate* gene has been cloned and characterized (PCT Publication WO 93/12141 dated June 24, 1993). However, prior to the present invention, despite attempts to achieve the same, no vertebrate *Serrate* gene was available.

Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *Serrate* genes (human *Serrate* and related genes of other species), and amino acid sequences of their encoded proteins, as well as derivatives (*e.g.*, fragments) and analogs thereof. Nucleic acids hybridizable to or complementary to the foregoing nucleotide sequences are also provided. In a specific embodiment, the Serrate protein is a human protein.

The invention relates to vertebrate Serrate derivatives and analogs of the invention which are functionally active, i.e., they are capable of displaying one or more known functional activities associated with a full-length (wild-type) Serrate protein. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with Serrate for binding) to an anti-Serrate antibody], immunogenicity (ability to generate antibody which binds to Serrate), ability to bind (or compete with Serrate for binding) to Notch or other toporythmic proteins or fragments thereof ("adhesiveness"), ability to bind (or compete with Serrate for binding) to a receptor for Serrate. "Toporythmic proteins" as used herein, refers to the protein products of *Notch*, *Delta*, *Serrate*, *Enhancer of split*, and *Deltex*, as well as other members of this interacting gene family which may be identified, *e.g.*, by virtue of the ability of their gene sequences to hybridize, or their homology to Delta, Serrate, or Notch, or the ability of their genes to display phenotypic interactions.

The invention further relates to fragments (and derivatives and analogs thereof) of vertebrate Serrate which comprise one or more domains of the Serrate protein, including but not limited to the intracellular domain, extracellular domain, transmembrane domain, membrane-

associated region, or one or more EGF-like (homologous) repeats of a Serrate protein, or any combination of the foregoing.

Antibodies to vertebrate Serrate, its derivatives and analogs, are additionally provided.

Methods of production of the vertebrate Serrate proteins, derivatives and analogs, *e.g.*, by recombinant means, are also provided.

The present invention also relates to therapeutic and diagnostic methods and compositions based on vertebrate Serrate proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: vertebrate Serrate proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the vertebrate Serrate proteins, analogs, or derivatives; and vertebrate Serrate antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or Serrate function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote Notch and/or Serrate function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localizati n of Notch and/or

S rrate prot in can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of a vertebrate Serrate which mediates binding to a Notch protein or a fragment thereof.

3.1. DEFINITIONS

As used herein, underscoring or italicizing the name of a gene shall indicate the gene, in contrast to its encoded protein product which is indicated by the name of the gene in the absence of any underscoring. For example, "*Serrate*" shall mean the *Serrate* gene, whereas "Serrate" shall indicate the protein product of the *Serrate* gene.

4. DESCRIPTION OF THE FIGURES

Figure 1. Nucleotide sequence (SEQ ID NO:1) and protein sequence (SEQ ID NO:2) of *Human Serrate-1* (also known as *Human Jagged-1 (HJ1)*).

Figure 2. "Complete" nucleotide sequence (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of *Human Serrate-2* (also known as *Human Jagged-2 (HJ2)*) generated on the computer by combining the sequence of clones pBS15 and pBS3-2 isolated from human fetal brain cDNA libraries. There is a deletion of approximately 120 nucleotides in the region of this sequence which encodes the portion of Human Serrat -2 between the signal sequence and the beginning of the DSL domain.

Figure 3. Nucleotide sequence (SEQ ID NO:5) of chick Serrate (*C-Serrate*) cDNA.

Figure 4. Amino acid sequence (SEQ ID NO:6) of C-Serrate (lacking the amino-terminus of the signal sequence). The putative cleavage site following the signal sequence (marking the predicted amino-terminus of the mature protein) is marked with an arrowhead; the DSL domain is indicat d by asterisks; the EGF-like rep ats (ELRs) ar

underlined with dash d lines; th cysteine rich region between th ELRs and the transmembrane domain is marked between arrows, and the single transmembrane domain (between amino acids 1042 and 1066) is shown in bold.

Figure 5. Alignment of the amino terminal sequences of *Drosophila melanogaster* Delta (SEQ ID NO:7) and Serrate (SEQ ID NO:8) with C-Serrate (SEQ ID NO:6). The region shown extends from the end of the signal sequence to the end of the DSL domain. The DSL domain is indicated. Identical amino acids in all three proteins are boxed.

Figure 6. Diagram showing the domain structures of *Drosophila* Delta and *Drosophila* Serrate compared with C-Serrate. The second cysteine-rich region just downstream of the EGF repeats, present only in C-Serrate and *Drosophila* Serrate, is not shown. Hydrophobic regions are shown in black; DSL domains are checkered and EGF-like repeats are hatched.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *Serrate* genes, and amino acid sequences of their encoded proteins. The invention further relates to fragments and other derivatives, and analogs, of vertebrate Serrate proteins. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. The invention provides vertebrate Serrate gen s and their encoded proteins of many different species. The Serrate genes of the invention include human *Serrate* and related genes (homologs) in vertebrate species. In specific embodiments, the Serrate genes and proteins are from mammals. In a preferred embodiment of the invention, the Serrate protein is a human protein. In most preferred embodiments, the Serrate protein is Human Serrate-1 or Human Serrate-2. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is provided.

Th invention relates to vertebrate Serrate derivatives and analogs of the invention which are

functionally active, i. ., they are capable of displaying one or mor known functional activities associat d with a full-length (wild-type) S rrate protein. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with Serrate for binding) to an anti-Serrate antibody], immunogenicity (ability to generate antibody which binds to Serrate), ability to bind (or compete with Serrate for binding) to Notch or other toporythmic proteins or fragments thereof ("adhesiveness"), ability to bind (or compete with Serrate for binding) to a receptor for Serrate. "Toporythmic proteins" as used herein, refers to the protein products of *Notch*, *Delta*, *Serrate*, *Enhancer of split*, and *Deltex*, as well as other members of this interacting gene family which may be identified, *e.g.*, by virtue of the ability of their gene sequences to hybridiz , or their homology to Delta, Serrate, or Notch, or the ability of their genes to display phenotypic interactions.

The invention further relates to fragments (and derivatives and analogs thereof) of a vertebrate Serrate which comprise one or more domains of the Serrate protein, including but not limited to the intracellular domain, extracellular domain, transmembrane domain, membrane-associated region, or one or more EGF-like (homologous) repeats of a Serrate protein, or any combination of the foregoing.

Antibodies to Serrate, its derivatives and analogs, are additionally provided.

As demonstrated *infra*, Serrate plays a critical role in development and other physiological processes, in particular, as a ligand to Notch, which is involved in cell fate (differentiation) determination. In particular, Serrate is believed to play a major role in determining cell fates in the central nervous system. The nucleic acid and amino acid sequences and antibodies thereto of the invention can be used for the detection and quantitation of Serrate mRNA and prot in of human and other species, to study expression ther of, to produce S rrate and fragments and other

derivatives and analogs thereof, in the study and manipulation of differentiation and other physiological processes. The pres nt invention also relates to therapeutic and diagnostic methods and compositions based on Serrate proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: vertebrate Serrate proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the vertebrate Serrate proteins, analogs, or derivatives; and vertebrate Serrate antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or Serrate function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote Notch and/or Serrate function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or Serrate protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of a vertebrate Serrate which mediates binding to a Notch protein or a fragment thereof.

The invention is illustrated by way of examples *infra* which disclos , *inter alia*, the cloning of a mouse *Serrate* homolog (S ction 6), the cloning of a *Xenopus* (frog) *Serrate* homolog (Section 7), the cloning of a chick *Serrate* homolog (Section 8), and the cloning of the human *Serrat* homologs *Human Serrate-1* (*HJ1*) and *Human Serrate-2* (*HJ2*) (Section 9).

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the sub-sections which follow.

5.1. ISOLATION OF THE *SERRATE* GENES

The invention relates to the nucleotide sequences of vertebrate *Serrate* nucleic acids. In specific embodiments, vertebrate *Serrate* nucleic acids comprise the cDNA sequences shown in Figure 1 (SEQ ID NO:1), Figure 2 (SEQ ID NO:3), Figure 3 (SEQ ID NO:6) or the coding regions thereof, or nucleic acids encoding a vertebrate Serrate protein (*e.g.*, having the sequence of SEQ ID NO:2, 4, or 6).

The invention provides nucleic acids consisting of at least 8 nucleotides (*i.e.*, a hybridizable portion) of a vertebrate *Serrate* sequence; in other embodiments, the nucleic acids consist of at least 10 (continuous) nucleotides, 25 nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a vertebrate *Serrate* sequence, or a full-length vertebrate *Serrate* coding sequence. The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a *Serrate* gene.

In a specific embodiment, a nucleic acid which is hybridizable to a vertebrate *Serrate* nucleic acid (*e.g.*, having sequence SEQ ID NO:1), or to a nucleic acid encoding a v rtebrat Serrate derivative, under conditions of low string ncy is provided. By way of example and not

limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreat d for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

In another specific embodiment, a nucleic acid which is hybridizable to a vertebrate Serrate nucleic acid under conditions of high stringency is provided. By way f example and not limitation, procedures using such conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Other conditions of high string ncy which may be used are well known in the art.

Nucleic acids encoding fragments and derivatives of vertebrate Serrate proteins (see Section 5.6), and vertebrate *Serrate* antisense nucleic acids (see Section 5.11) are additionally provided. As is readily apparent, as used herein, a "nucleic acid encoding a fragment or portion of a Serrate protein" shall be construed as referring to a nucleic acid encoding only the recited fragment or portion of the Serrate protein and not the other contiguous portions of the Serrate protein as a continuous sequence.

Fragments of vertebrate *Serrate* nucleic acids comprising regions of homology to other toporythmic proteins are also provided. The DSL regions (regions of homology with *Drosophila* Delta and Serrate) of Serrate proteins of other species are also provided. Nucleic acids encoding conserved regions between Delta and Serrate, such as those represented by Serrate amino acids 63-73, 124-134, 149-158, 195-206, 214-219, and 250-259 of SEQ ID NO:8, or by the DSL domains are also provided.

Specific embodiments for the cloning of a vertebrate *Serrate* gene, presented as a particular example but not by way of limitation, follows:

For expression cloning (a technique commonly known in the art), an expression library is constructed by methods known in the art. For example, mRNA (*e.g.*, human) is isolated, cDNA is made and ligated into an expression vector (*e.g.*, a bacteriophage derivative) such that it is capable of being expressed by the host cell into which it is then introduced. Various screening assays can then be used to select for the expressed Serrate product. In one embodiment, anti-Serrate antibodies can be used for selection.

In another preferred aspect, PCR is used to amplify the desired sequence in a genomic or cDNA library, prior to selection. Oligonucleotide primers representing known Serrate sequences can be used as primers in PCR. In a preferred aspect, the oligonucleotide primers encode at least part of the Serrate conserved segments of strong homology between *Serrate* and *Delta*. The synthetic oligonucleotides

may be utilized as primers to amplify by PCR sequences from a source (RNA or DNA), preferably a cDNA library, of potential interest. PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycl r and Taq polymerase (Gen Amp™). The DNA being amplified can include mRNA or cDNA or genomic DNA from any eukaryotic species. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the known *Serrate* nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred. For same species hybridization, moderately stringent conditions are preferred. After successful amplification of a segment of a *Serrate* homolog, that segment may be cloned and sequenced, and utilized as a probe to isolate a complete cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and th production of its protein product for functional analysis, as described *infra*. In this fashion, additional genes encoding Serrate proteins may be identified. Such a procedure is presented by way of example in various examples sections *infra*.

The above-methods are not meant to limit the following general description of methods by which clones of vertebrate *Serrate* may be obtained.

Any vertebrate cell potentially can serve as the nucleic acid source for the molecular cloning of the *Serrate* gene. The nucleic acid sequences encoding Serrate can be isolated from human, porcine, bovine, feline, avian, equine, canine, as well as additional primate sources, etc. For example, we have amplified fragments of the appropriate size in mouse, *Xenopus*, and human, by PCR using cDNA libraries with *Drosophila S rrate* primers. The DNA may be obtained by standard procedures known in the art from clon d DNA (.g., a

DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments th reof, purified from the d sired cell. (See, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Appr ach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but n t limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a number of ways. For example, if a *Serrate* (of any species) gene or its specific RNA, or a fragment thereof, *e.g.*, an extracellular domain (see Section 5.6), is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, Science 196:180; Grunstein, M. And Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available. Further

selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, *e.g.*, has similar or identical electrophoretic migration, isolectric focusing behavior, proteolytic digestion maps, receptor binding activity, *in vitro* aggregation activity ("adhesiveness") or antigenic properties as known for Serrate. If an antibody to Serrate is available, the Serrate protein may be identified by binding of labeled antibody to the putatively Serrate synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

The *Serrate* gene can also be identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified *Serrate* DNA of another species (*e.g.*, human, chick). Immunoprecipitation analysis or functional assays (*e.g.*, aggregation ability *in vitro*; binding to receptor; see *infra*) of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against Serrate protein. A radiolabeled *Serrate* cDNA can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabeled mRNA or cDNA may then be used as a probe to identify the *Serrate* DNA fragments from among other genomic DNA fragments.

Alternatives to isolating the *Serrate* genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the Serrate protein. For example, RNA

for cDNA cloning of the *Serrate* gene can be isolated from cells which express *Serrate*. Other methods are possible and within the scope of the invention.

The identified and isolated gene can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and *Serrate* gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionization, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated *Serrate* gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from

the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The Serrate sequences provided by the instant invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native Serrate proteins, and those encoded amino acid sequences with functionally equivalent amino acids, all as described in Section 5.6 *infra* for Serrate derivatives.

5.2. EXPRESSION OF THE *SERRATE* GENES

The nucleotide sequence coding for a vertebrate Serrate protein or a functionally active fragment or other derivative thereof (see Section 5.6), can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native vertebrate *Serrate* gene and/or its flanking regions. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In a specific embodiment, the adhesive portion of the *Serrate* gene is expressed. In other specific embodiments, a *Human Serrate* gene or a sequence encoding a functionally active portion of a human Serrate gene, such as *Human Serrate-1 (HJ2)* or *Human Serrate-2 (HJ2)*, is expressed. In yet another embodiment, a fragment of Serrate comprising the extracellular domain, or other derivative, or analog of Serrate is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a Serrate protein or peptide fragment may be regulated by a second nucleic acid sequence so that the Serrate protein or peptid is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a Serrate protein may be controlled by any promoter/enhancer element known in th art. Promoters which may be used to control toporythmic gene expression include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, th ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycer l kinas) promoter, alkaline phosphatase prom ter, and the following animal transcriptional control

regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, C ll 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Expression vectors containing *Serrate* gene inserts can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted toporythmic gene. In the second

approach, the recombinant vector/host system can be identified and select d based upon the presence or abs nc of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. F r example, if the *Serrate* gene is inserted within the marker gene sequence of the vector, recombinants containing the *Serrate* insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the *Serrate* gene product *in vitro* assay systems, *e.g.*, aggregation (binding) with Notch, binding to a receptor, binding with antibody.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered Serrate protein may be controlled. Furth rmore, different host cells hav characteristic and specific mechanisms for the translational and post-translational processing and modificati n (*e.g.*,

glycosylation, cl avage [e.g., of signal sequence]) of proteins. Appropriate cell lines or host systems can be chosen to ensur the desired modification and processing of the foreign protein express d. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous mammalian toporythmic protein. Furthermore, different vector/host expression systems may effect processing reactions such as proteolytic cleavages to different extents.

In other specific embodiments, the Serrate protein, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

5.3. IDENTIFICATION AND PURIFICATION OF THE *SERRATE* GENE PRODUCTS

In particular aspects, the invention provides amino acid sequences of a vertebrate Serrate, preferably a human Serrate homolog, and fragments and derivatives thereof which comprise an antigenic determinant (*i*.e., can be recognized by an antibody) or which are otherwise functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" material as used herein refers to that material displaying one r more known functional activiti s associated with a full-length (wild-type) Serrate protein,

e.g., binding to Notch or a portion thereof, binding to any other Serrate ligand, antigenicity (binding to an anti-S rrate antibody), etc.

In specific embodiments, the invention provides fragments of a vertebrate Serrate protein consisting of at least 6 amino acids, 10 amino acids, 25 amino acids, 50 amino acids, or of at least 75 amino acids. In other embodiments, the proteins comprise or consist essentially of an extracellular domain, DSL domain, epidermal growth factor-like repeat (ELR) domain, one or any combination of ELRs, cysteine-rich region, transmembrane domain, or intracellular (cytoplasmic) domain, or a portion which binds to Notch, or any combination of the foregoing, of a Serrate protein. Fragments, or proteins comprising fragments, lacking some or all of the foregoing regions of a vertebrate Serrate prot in are also provided. Nucleic acids encoding the foregoing are provided.

Once a recombinant which expresses the vertebrate *Serrate* gene sequence is identified, the gene product can be analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive labelling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

Once the Serrate protein is identified, it may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay (see Section 5.7).

Alternatively, once a Serrate protein produced by a recombinant is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the chimeric gene contained in the recombinant. As a result, the protein can be synth sized by standard chemical methods known in the art (*e.g.*, see Hunkapiller, M., et al., 1984, Nature 310:105-111).

In a specific embodiment of the present invention, such Serrate proteins, whether produced by recombinant DNA techniques or by chemical synthetic methods, include but are not limited to those containing, as a primary amino acid sequence, all or part of the amino acid sequence substantially as depicted in Figures 1, 2, or 3 (SEQ ID NO:2, 4, or 6, respectively), as well as fragments and other derivatives, and analogs thereof.

5.4. STRUCTURE OF THE *SERRATE* GENES AND PROTEINS

The structure of the *Serrate* genes and proteins can be analyzed by various methods known in the art.

5.4.1. GENETIC ANALYSIS

The cloned DNA or cDNA corresponding to the vertebrate *Serrate* gene can be analyzed by methods including but not limited to Southern hybridization (Southern, E.M., 1975, J. Mol. Biol. 98:503-517), Northern hybridization (see *e.g.*, Freeman et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:4094-4098), restriction endonuclease mapping (Maniatis, T., 1982, Molecular Cloning, A Laboratory, Cold Spring Harbor, New York), and DNA sequence analysis. Polymerase chain reaction (PCR; U.S. Patent Nos. 4,683,202, 4,683,195 and 4,889,818; Gyllenstein et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7652-7656; Ochman et al., 1988, Genetics 120:621-623; Loh et al., 1989, Science 243:217-220) followed by Southern hybridization with a *Serrate*-specific probe can allow the detection of the *Serrate* gene in DNA from various cell types. Methods of amplification other than PCR are commonly known and can also be employed. In one embodiment, Southern hybridization can be used to determine the genetic linkage of *Serrate*. Northern hybridization analysis can be used to determine the expression of the *Serrate* gene. Various cell types, at various states of development or activity can be tested for *Serrate* expression. Examples of such techniques and their results are described in Section 6, *infra*. The stringency of the hybridization conditions for

both Southern and Northern hybridization can be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific *Serrate* probe used.

Restriction endonuclease mapping can be used to roughly determine the genetic structure of the *Serrate* gene. In a particular embodiment, cleavage with restriction enzymes can be used to derive the restriction map shown in Figure 2, *infra*. Restriction maps derived by restriction endonucleas cleavage can be confirmed by DNA sequence analysis.

DNA sequence analysis can be performed by any techniques known in the art, including but not limited to the method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger, F., et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699), or use of an automated DNA sequenator (*e.g.*, Applied Biosystems, Foster City, CA). The cDNA sequence of a representative Serrate gene comprises the sequence substantially as depicted in Figures 1 and 2, and is described in Section 9, *infra*.

5.4.2. PROTEIN ANALYSIS

The amino acid sequence of the Serrate proteins can be derived by deduction from the DNA sequence, or alternatively, by direct sequencing of the protein, *e.g.*, with an automated amino acid sequencer. The amino acid sequence of a representative Serrate protein comprises the sequence substantially as depicted in Figure 1, and detailed in Section 9, *infra*, with the representative mature prot in that shown by amino acid numbers 30-1219.

The Serrate protein sequence can be further characterized by a hydrophilicity analysis (Hopp, T. and Woods, K., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824). A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of the Serrate protein and th corresponding regions of the gene sequence which encode such regions.

Secondary, structural analysis (Chou, P. and Fasman, G., 1974, Biochemistry 13:222) can also be don , to identify r gions of Serrate that assume specific s condary structures.

Manipulation, translation, and secondary structure prediction, as well as open reading frame prediction and plotting, can also be accomplished using computer software programs available in the art.

Other methods of structural analysis can also be employed. These include but are not limited to X-ray crystallography (Engstom, A., 1974, Biochem. Exp. Biol. 11:7-13) and computer modeling (Fletterick, R. and Zoller, M. (eds.), 1986, Computer Graphics and Molecular Modeling, *in* Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

5.5. GENERATION OF ANTIBODIES TO SERRATE PROTEINS AND DERIVATIVES THEREOF

According to the invention, a vertebrate Serrate protein, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which recognize such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to human Serrate are produced. In another embodiment, antibodies to the extracellular domain of Serrate are produced. In another embodiment, antibodies to the intracellular domain of Serrate are produced.

Various procedures known in the art may be used for the production of polyclonal antibodies to a Serrate protein or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the Serrate protein encoded by a sequence depicted in Figure 1, or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native Serrate protein, or a synthetic v rsion, or derivative

(.g., fragment) thereof, including but not limited to rabbits, mice, rats, tc. Various adjuvants may be used to incr ase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

For preparation of monoclonal antibodies directed toward a vertebrate Serrate protein sequence or analog thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for Serrate together with genes from a human antibody molecule of appropriate biol gical activity

can be used; such antib dies are within the scope of this inv ntion.

According to the inv ntion, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce Serrate-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for Serrate proteins, derivatives, or analogs.

Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of a Serrate protein, one may assay generated hybridomas for a product which binds to a Serrate fragment containing such domain. For selection of an antibody specific to vertebrate (*e.g.*, human) Serrate, one can select on the basis of positive binding to vertebrate Serrate and a lack of binding to *Drosophila* Serrate. In another embodiment, one can select for binding to human Serrate and not to Serrate of other species.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the protein sequences of the invention (*e.g.*, see Section 5.7, *infra*), *e.g.*, for imaging th se proteins, measuring

levels thereof in appropriate physiological samples, in diagnostic methods, etc.

Antibodies specific to a domain of a Serrate protein are also provided. In a specific embodiment, antibodies which bind to a Notch-binding fragment of Serrate are provided.

In another embodiment of the invention (see *infra*), anti-Serrate antibodies and fragments thereof containing the binding domain are Therapeutics.

5.6. SERRATE PROTEINS, DERIVATIVES AND ANALOGS

The invention further relates to vertebrate Serrate proteins, and derivatives (including but not limited to fragments) and analogs of Serrate proteins. Nucleic acids encoding vertebrate Serrate protein derivatives and protein analogs are also provided. In one embodiment, the Serrate proteins are encoded by the vertebrate Serrate nucleic acids described in Section 5.1 *supra*. In particular aspects, the proteins, derivatives, or analogs are of frog, mouse, rat, pig, cow, dog, monkey, or human Serrate proteins.

The production and use of derivatives and analogs related to vertebrate Serrate are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, i.e., capable of exhibiting one or more functional activities associated with a full-length, wild-type Serrate protein. As one example, such derivatives or analogs which have the desired immunogenicity or antigenicity can be used, for example, in immunoassays, for immunization, for inhibition of Serrate activity, etc. Such molecules which retain, or alternatively inhibit, a desired Serrate property, *e.g.*, binding to Notch or other toporythmic proteins, binding to a cell-surface receptor, can be used as inducers, or inhibitors, respectively, of such property and its physiological correlates. A specific embodiment relates to a Serrate fragment that can be bound by an anti-Serrat antibody but cannot bind to a Notch prot in or other toporythmic protein. Derivatives or anal gs of

Serrate can be test d for the desired activity by procedures known in the art, including but not limited to th assays describ d in Section 5.7.

In particular, Serrate derivatives can be made by altering *Serrate* sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, oth r DNA sequences which encode substantially the same amino acid sequence as a *Serrate* gene may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of *Serrate* genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the Serrate derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a Serrate protein including altered sequences in which functionally equivalent amino acid residues are substitut d for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a specific embodiment of the invention, proteins consisting of or comprising a fragment of a vertebrate Serrate protein consisting of at least 10 (continuous) amino

acids of the Serrate protein is provided. In other embodiments, th fragment consists of at least 20 or 50 amino acids of th Serrate protein. In specific embodiments, such fragments are not larger than 35, 100 or 200 amino acids. Derivatives or analogs of vertebrate Serrate include but ar not limited to those peptides which are substantially homologous to a vertebrate Serrate or a fragment thereof (*e.g.*, at least 30% identity over an amino acid sequence of identical size) or whose encoding nucleic acid is capable of hybridizing to a coding vertebrate *Serrate* sequence.

The Serrate derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned *Serrate* gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of Serrate, care should be taken to ensure that the modified gene remains within the same translational reading frame as *Serrate*, uninterrupted by translational stop signals, in the gene region where the desired Serrate activity is encoded.

Additionally, the Serrate-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem 253:6551), use of TAB® linkers (Pharmacia), etc.

Manipulations f the Serrat sequence may also be made at the protein level. Included within the scope of the invention are Serrate protein fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any f numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives of Serrate can be chemically synthesized. For example, a peptide corresponding to a portion of a Serrate protein which comprises the desired domain (see Section 5.6.1), or which mediates the desired aggregation activity *in vitro*, or binding to a receptor, can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the Serrate sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids.

In a specific embodiment, the Serrate derivative is a chimeric, or fusion, protein comprising a vertebrate Serrate protein or fragment thereof (preferably consisting of at least a domain or motif of the Serrate protein, or at least 10 amino acids of the Serrate protein) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment,

such a chimeric prot in is produced by recombinant expression of a nucleic acid encoding the protein (comprising a Serrate-coding sequenc joined in-fram to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing th chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. In a specific embodiment, a chimeric nucleic acid encoding a mature vertebrate Serrate protein with a heterologous signal sequence is expressed such that the chimeric protein is expressed and processed by the cell to the mature Serrate protein. As another example, and not by way of limitation, a recombinant molecule can be constructed according to the invention, comprising coding portions of both *Serrate* and another toporythmic gene, *e.g.*, Delta. The encoded prot in of such a recombinant molecule could exhibit properties associated with both Serrate and Delta and portray a novel profile of biological activities, including agonists as well as antagonists. The primary sequence of Serrate and Delta may also be used to predict tertiary structure of the molecules using computer simulation (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828); *Serrate/Delta* chimeric recombinant genes could be designed in light of correlations between tertiary structure and biological function. Likewise, chimeric genes comprising portions of a vertebrate *Serrate* fused to any heterologous protein-encoding sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of a vertebrate Serrate of at least ten amino acids.

In another specific embodiment, the Serrate derivative is a fragment of Serrate comprising a region of homology with another toporythmic protein. As used herein, a r gion of a first prot in shall be considered "homologous" to a second protein when the amino acid sequ nce of the region

is at least 30% identical or at least 75% either identical or involving conservative changes, when compared to any sequence in the second protein of an equal number of amino acids as the number contained in the region. For example, such a Serrate fragment can comprise one or more regions homologous to Delta, or DSL domains or portions thereof.

Other specific embodiments of derivatives and analogs are described in the subsections below and examples sections *infra*.

5.6.1. DERIVATIVES OF SERRATE CONTAINING ONE OR MORE DOMAINS OF THE PROTEIN

In a specific embodiment, the invention relates to vertebrate Serrate derivatives and analogs, in particular vertebrate Serrate fragments and derivatives of such fragments, that comprise, or alternatively consist of, one or more domains of the Serrate protein, including but not limited to the extracellular domain, DSL domain, ELR domain, cysteine rich domain, transmembrane domain, intracellular domain, membrane-associated region, and one or more of the EGF-like repeats (ELR) of the Serrate protein, or any combination of the foregoing. In particular examples relating to the human and chick Serrate proteins, such domains are identified in Examples Section 9 and 8, respectively.

In a specific embodiment, the molecules comprising specific fragments of vertebrate Serrate are those comprising fragments in the respective Serrate protein most homologous to specific fragments of the *Drosophila* Serrate and/or Delta proteins. In particular embodiments, such a molecule comprises or consists of the amino acid sequences homologous to SEQ ID NO:10, 12, or 18. Alternatively, a fragment comprising a domain of a Serrate homolog can be identified by protein analysis methods as described in Section 5.3.2.

5.6.2. DERIVATIVES OF SERRATE THAT MEDIATE BINDING TO TOPORYTHMIC PROTEIN DOMAINS

The inv ntion also provides for vertebrat Serrate fragments, and analogs or derivatives of such fragments, which mediate binding to toporythmic proteins (and thus are termed herein "adhesive"), and nucleic acid sequences encoding the foregoing.

In a specific embodiment, the adhesive fragment of Serrate is that comprising the portion of Serrate most homologous to about amino acid numbers 85-283 or 79-282 of the *Drosophila* Serrate sequence (see PCT Publication WO 93/12141 dated June 24, 1993).

In a particular embodiment, the adhesive fragment of a Serrate protein comprises the DSL domain, or a portion thereof. Subfragments within the DSL domain that mediate binding to Notch can be identified by analysis of constructs expressing deletion mutants.

The ability to bind to a toporythmic protein (preferably Notch) can be demonstrated by *in vitro* aggregation assays with cells expressing such a toporythmic protein as well as cells expressing Serrate or a Serrate derivative (See Section 5.7). That is, the ability of a Serrate fragment to bind to a Notch protein can be demonstrated by detecting the ability of the Serrate fragment, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

The nucleic acid sequences encoding toporythmic proteins or adhesive domains thereof, for use in such assays, can be isolated from human, porcine, bovine, feline, avian, equine, canine, or insect, as well as primate sources and any other species in which homologs of known toporythmic genes can be identified.

5.7. ASSAYS OF SERRATE PROTEINS, DERIVATIVES AND ANALOGS

The functional activity of vertebrate Serrat proteins, derivatives and analogs can b assayed by various methods.

For example, in one embodiment, where one is assaying for the ability to bind or compete with wild-type Serrate for binding to anti-Serrate antibody, various immunoassays known in the art can be used, including but not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where one is assaying for the ability to mediate binding to a toporythmic protein, *e.g.*, Notch, one can carry out an *in vitro* aggregation assay such as described in PCT Publication WO 93/12141 dated June 24, 1993 (see also Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699).

In another embodiment, where a receptor for Serrate is identified, receptor binding can be assayed, *e.g.*, by means well-known in the art. In another embodiment, physiological correlates of Serrate binding to cells

expressing a Serrat receptor (signal transduction) can be assayed.

In another embodiment, in insect or other model systems, genetic studies can be done to study the phenotypic effect of a Serrate mutant that is a derivative or analog of wild-type vertebrate *Serrate*.

Other methods will be known to the skilled artisan and are within the scope of the invention.

5.8. THERAPEUTIC USES

The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: vertebrat Serrate proteins and analogs and derivatives (including fragments) thereof (*e.g.*, as described hereinabove); antibodies thereto (as described hereinabove); nucleic acids encoding the vertebrate Serrate proteins, analogs, or derivatives (*e.g.*, as described hereinabove); and *Serrate* antisense nucleic acids. As stated *supra*, the Antagonist Therapeutics of the invention are those Therapeutics which antagonize, or inhibit, a vertebrate Serrate function and/or Notch function (since Serrate is a Notch ligand). Such Antagonist Therapeutics are most preferably identified by use of known convenient *in vitro* assays, *e.g.*, based on their ability to inhibit binding of Serrate to another protein (*e.g.*, a Notch protein), or inhibit any known Notch or Serrate function as preferably assayed *in vitro* or in cell culture, although genetic assays (*e.g.*, in *Drosophila*) may also be employed. In a preferred embodiment, the Antagonist Therapeutic is a protein or derivative thereof comprising a functionally active fragment such as a fragment of Serrate which mediates binding to Notch, or an antibody thereto. In other specific embodiments, such an Antagonist Therapeutic is a nucl ic acid capable of expressing a molecule comprising a fragment of Serrate which binds to Notch, or a *Serrate* antisense nucleic acid (see Section 5.11 herein). It should

be not d that preferably, suitable *in vitro* or *in vivo* assays, as described *infra*, should be utilized to determine the eff ct of a specific Therap utic and wheth r its administration is indicated for treatment of the affected tissue, since the developmental history of the tissue may determine whether an Antagonist or Agonist Therapeutic is desired.

In addition, the mode of administration, *e.g.*, whether administered in soluble form or administered via its encoding nucleic acid for intracellular recombinant expression, of the Serrate protein or derivative can affect whether it acts as an agonist or antagonist.

In another embodiment of the invention, a nucleic acid containing a portion of a vertebrate *Serrate* gene is used, as an Antagonist Therapeutic, to promote *Serrate* inactivation by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

The Agonist Therapeutics of the invention, as described *supra*, promote Serrate function. Such Agonist Therapeutics include but are not limited to proteins and derivatives comprising the portions of Notch that mediate binding to Serrate, and nucleic acids encoding the foregoing (which can be administered to express their encoded products *in vivo*).

Further descriptions and sources of Therapeutics of the inventions are found in Sections 5.1 through 5.7 herein.

Molecules which retain, or alternatively inhibit, a desired Serrate property, *e.g.*, binding to Notch, binding to an intracellular ligand, can be used therapeutically as inducers, or inhibitors, respectively, of such property and its physiological correlates. In a specific embodiment, a peptide (*e.g.*, in the range of 10-50 or 15-25 amino acids; and particularly of about 10, 15, 20 or 25 amino acids) containing the sequence of a portion of a vertebrate Serrate which binds to Notch is used to antagonize Notch function. In a specific embodiment, such an Antagonist Therapeutic is

used to treat or prevent human or other malignancies associated with incr ased Notch expression (*e.g.*, cervical cancer, colon cancer, breast cancer, squamous adenocarcimas (see *infra*)). Derivatives or analogs of Serrate can be tested for the desired activity by procedures known in th art, including but not limited to the assays described in the examples *infra*. For example, molecules comprising vertebrate Serrate fragments which bind to Notch EGF-repeats (ELR) 11 and 12 and which are smaller than a DSL domain, can be obtained and selected by expressing deletion mutants and assaying for binding of the expressed product to Notch by any of the several methods (*e.g.*, *in vitro* cell aggregation assays, interaction trap system), some of which are described in the Examples Sections *infra*. In one specific embodiment, peptide libraries can be screened to select a peptide with the desired activity; such screening can be carried out by assaying, *e.g.*, for binding to Notch or a molecule containing the Notch ELR 11 and 12 repeats.

The Agonist and Antagonist Therapeutics of the invention have therapeutic utility for disorders of cell fate. The Agonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an absence or decreased (relative to normal, or desired) levels of Notch or Serrate function, for example, in patients where Notch or Serrate protein is lacking, genetically defective, biologically inactive or underactive, or underexpressed; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays (see *infra*) indicate the utility of Serrate agonist administration. The absence or decreased levels in Notch or Serrate function can be readily detected, *e.g.*, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for protein levels, structure and/or activity of the expressed Notch or Serrate protein. Many methods standard in the art can be thus employed, including but not limited to immunoassays to detect and/or visualize Notch or Serrate protein (*e.g.*, Western blot, immunoprecipitation followed by

sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect Notch or Serrate expression by detecting and/or visualizing r spectiv ly Notch or Serrate mRNA (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.)

In vitro assays which can be used to determine whether administration of a specific Agonist Therapeutic or Antagonist Therapeutic is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant cells (*e.g.*, by promoting terminal differentiation) is selected for therapeutic use *in vivo*. Many assays standard in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, *fos*, *myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc. In a specific aspect, the malignant cell cultures are separately exposed to (1) an Agonist Therapeutic, and (2) an Antagonist Therapeutic; the result of the assay can indicate which type of Therapeutic has therapeutic efficacy.

In another embodiment, a Therapeutic is indicated for use which exhibits the desired effect, inhibition or promotion of cell growth, upon a patient cell sample from tissue having or suspected of having a hyper- or hypoproliferative disorder, respectively. Such hyper- or hypoproliferative disorders include but are not limited to those described in Sections 5.8.1 through 5.8.3 *infra*.

In another specific embodiment, a Therapeutic is indicated for use in treating nerve injury or a nervous system degenerative disorder (see Section 5.8.2) which exhibits *in vitro* promotion of nerve regeneration/neurite extension from nerve cells of the affected patient type.

In addition, administration of an Antagonist Therapeutic of the invention is also indicated in diseases or disorders determined or known to involve a Notch or Serrat dominant activated phenotype ("gain of function" mutations.) Administration of an Agonist Therapeutic is indicated in diseases or disorders determined or known to involve a Notch or Serrate dominant negative phenotype ("loss of function" mutations). The functions of various structural domains of the Notch protein have been investigated *in vivo*, by ectopically expressing a series of *Drosophila Notch* deletion mutants under the hsp70 heat-shock promoter, as well as eye-specific promoters (see Rebay et al., 1993, Cell 74:319-329). Two classes of dominant phenotypes were observed, one suggestive of *Notch* loss-of function mutations and the other of *Notch* gain-of-function mutations. Dominant "activated" phenotypes resulted from overexpression of a protein lacking most extracellular sequences, while dominant "negative" phenotypes resulted from overexpression of a protein lacking most intracellular sequences. The results indicated that Notch functions as a receptor whose extracellular domain mediates ligand-binding, resulting in the transmission of developmental signals by the cytoplasmic domain. We have shown that Serrate binds to the Notch ELR 11 and 12 (see PCT Publication WO 93/12141).

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more

normal (*i.e.*, less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed ph notyp) is select d for therapeutic use. Many assays standard in the art can b used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present. For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic or pre-neoplastic disorder desired to be treated or prevented, or is derived from the neural or other cell type upon which an effect is desired, according to the present invention.

The Antagonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving increased (relative to normal, or desired) levels of Notch or Serrate function, for example, where the Notch or Serrate protein is overexpressed or overactive; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays indicate the utility of Serrate antagonist administration. The increased levels of Notch or Serrate function can be readily detected by methods such as those described above, by quantifying protein and/or RNA. *In vitro* assays with cells of patient tissue sample or the appropriat cell line or cell type, to determine therapeutic utility, can be carried out as described above.

5.8.1. MALIGNANCIES

Malignant and pre-neoplastic conditions which can be t sted as d scribed *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to those described below in Sections 5.8.1 and 5.9.1.

Malignancies and related disorders, cells of which type can be tested *in vitro* (and/or *in vivo*), and upon observing the appropriate assay result, treated according to the present invention, include but are not limited to those listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

- Leukemia
  - acute leukemia
    - acute lymphocytic leukemia
    - acute myelocytic leukemia
      - myeloblastic
      - promyelocytic
      - myelomonocytic
      - monocytic
      - erythroleukemia
  - chronic leukemia
    - chronic myelocytic (granulocytic) leukemia
    - chronic lymphocytic leukemia
- Polycythemia vera
- Lymphoma
  - Hodgkin's disease
  - non-Hodgkin's disease
- Multiple myeloma
- Waldenström's macroglobulinemia
- Heavy chain disease
- Solid tumors
  - sarcomas and carcinomas
    - fibrosarcoma
    - myxosarcoma
    - liposarcoma
    - chondrosarcoma
    - ost ogenic sarcoma
    - chordoma

angiosarcoma
ndotheliosarcoma
lymphangiosarcoma
lymphangioendotheliosarcoma
synovioma
mesothelioma
Ewing's tumor
leiomyosarcoma
rhabdomyosarcoma
colon carcinoma
pancreatic cancer
breast cancer
ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
menangioma
melanoma
neuroblastoma
retinoblastoma

In specific embodiments, malignancy or dysproliferative changes (such as m taplasias and dysplasias) are treated or pr vented in epithelial tissues such as those in the cervix, esophagus, and lung.

Malignancies of the colon and cervix exhibit increased expression of human Notch relative to such non-malignant tissue (see PCT Publication no. WO 94/07474 published April 14, 1994, incorporated by reference herein in its entirety). Thus, in specific embodiments, malignancies or premalignant changes of the colon or cervix are treated or prevented by administering an effective amount of an Antagonist Therapeutic, *e.g.*, a Serrate derivative, that antagonizes Notch function. The presence of increased Notch expression in colon, and cervical cancer suggests that many more cancerous and hyperproliferative conditions exhibit upregulated Notch. Thus, in specific embodiments, various cancers, *e.g.*, breast cancer, squamous adenocarcinoma, seminoma, melanoma, and lung cancer, and premalignant changes therein, as well as other hyperproliferative disorders, can be treated or prevented by administration of an Antagonist Therapeutic that antagonizes Notch function.

5.8.2. NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

(i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;

(ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;

(iii) malignant lesions, in which a portion of th nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue;

(iv) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;

(v) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

(vi) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami dis ase (primary

degeneration of the corpus callosum), and alcoholic cereb llar degeneration;

(vii) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;

(viii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

(ix) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons (see also Section 5.8). For example, and not by way of limitation, Therapeutics which elicit any of the following effects may b useful according to the invention:

(i) increased survival time of neurons in cultur ;

(ii) increased sprouting of neurons in culture or *in vivo*;

(iii) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

(iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of n urons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of

neur ns may be d tected by meth ds set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

In a specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.8.3. TISSUE REPAIR AND REGENERATION

In another embodiment of the invention, a Therapeutic of the invention is used for promotion of tissue regeneration and repair, including but not limited to treatment of benign dysproliferative disorders. Specific embodiments are directed to treatment of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive prolif ration of the skin and delay in proper cell fate determination), and baldness (a condition in which terminally

differentiated hair follicles (a tissue rich in Notch) fail to function properly). In another embodiment, a Therapeutic of the invention is used to treat degenerative or traumatic disorders of the sensory epithelium of the inner ear.

5.9. PROPHYLACTIC USES

5.9.1. MALIGNANCIES

The Therapeutics of the invention can be administered to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such administration is indicated where the Therapeutic is shown in assays, as described *supra*, to have utility for treatment or prevention of such disorder. Such prophylactic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic

irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyp rplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. As mentioned *supra*, such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degre kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*,

familial polyposis of th colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, an Antagonist Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, or cervical cancer.

5.9.2. OTHER DISORDERS

In other embodiments, a Therapeutic of the invention can be administered to prevent a nervous system disorder described in Section 5.8.2, or other disorder (*e.g.*, liver cirrhosis, psoriasis, keloids, baldness) described in Section 5.8.3.

5.10. DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

The Therapeutics of the invention can be tested *in vivo* for the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

5.11. ANTISENSE REGULATION OF *SERRATE* EXPRESSION

The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six or of at least ten nucleotides that are antisense to a gene or cDNA encoding a vertebrate Serrate or a portion thereof.

"Antisense" as used herein refers to a nucleic acid capable of hybridizing to a portion of a vertebrate *Serrate* RNA (preferably mRNA) by virtue of some sequence complementarity. Such antisense nucleic acids have utility as Antagonist Therapeutics of the invention, and can be used in the treatment or prevention of disorders as described *supra* in Section 5.8 and its subsections.

The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

In a specific embodiment, the *Serrate* antisense nucleic acids provided by the instant invention can be used for the treatment of tumors or other disorders, the cells of which tumor type or disorder can be demonstrated (*in vitro* or *in vivo*) to express a *Serrate* gene or a *Notch* gene. Such demonstration can be by detection of RNA or of protein.

The invention further provides pharmaceutical compositions comprising an effective amount of the *Serrate* antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *infra* in Section 5.12. Methods for treatment and prevention of disorders (such as those described in Sections 5.8 and 5.9) comprising administering the pharmaceutical compositions of the invention are also provided.

In another embodiment, the invention is directed to methods for inhibiting the expression of a *Serrate* nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense vertebrate *Serrate* nucleic acid of the invention.

Serrate antisense nucleic acids and their uses are described in detail below.

5.11.1. *VERTEBRATE SERRATE* ANTISENSE NUCLEIC ACIDS

The vertebrate *Serrate* antisense nucleic acids are of at l ast six nucleotides and are preferably oligonucleotides (ranging preferably from 10 to about 50 oligonucleotides). In specific aspects, the oligonucleotide contains at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides antisense to a *Serrate* gene. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a vertebrate *Serrate* antisense oligonucleotide is provided, preferably of single-stranded DNA. In a most preferred aspect, such an oligonucleotide comprises a sequence antisense to the sequence encoding an SH3 binding domain or a Notch-binding domain of Serrate, most preferably, of a human Serrate homolog. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The Serrate antisense oligonucleotide may comprise at least one modified base moiety which is selected from th group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosin , 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine,

5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanin , 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially

availabl from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotid s may be synthesized by th method of St in et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

In a specific embodiment, the *Serrate* antisense oligonucleotide comprises catalytic RNA, or a ribozyme (se , e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225). In another embodiment, the oligonucleotide is a 2′-0-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

In an alternative embodiment, the *Serrate* antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the *Serrate* antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the Serrate antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the h rpes thymidine kinase promoter (Wagner et al.,

1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript specific to a vertebrate *Serrate* gene, preferably a human *Serrate* gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded Serrate antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a *Serrate* RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

5.11.2. THERAPEUTIC UTILITY OF VERTEBRATE *SERRATE* ANTISENSE NUCLEIC ACIDS

The vertebrate *Serrate* antisense nucleic acids can be used to treat (or prevent) malignancies or other disorders, of a cell type which has been shown to express *Serrate* or *Notch*. In specific embodiments, the malignancy is cervical, breast, or colon cancer, or squamous adenocarcinoma. Malignant, neoplastic, and pre-neoplastic cells which can be tested for such expression include but are not limited to those described *supra* in Sections 5.8.1 and 5.9.1. In a preferred embodiment, a single-stranded DNA antisense Serrate oligonucleotide is used.

Malignant (particularly, tumor) cell types which express *Serrate* or *Notch* RNA can be identified by various

methods known in the art. Such methods include but are not limit d to hybridization with a *Serrate* or *Notch*-specific nucleic acid (*e.g.* by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into Notch or Serrate, immunoassay, etc. In a preferred aspect, primary tumor tissue from a patient can be assayed for N tch or Serrate expression prior to treatment, *e.g.*, by immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention (see Section 5.12), comprising an effective amount of a vertebrate *Serrate* antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a patient having a malignancy which is of a type that expresses *Notch* or *Serrate* RNA or protein.

The amount of *Serrate* antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity of the tumor type to be treated *in vitro*, and then in useful animal model systems prior to testing and use in humans.

In a specific embodiment, pharmaceutical compositions comprising vertebrate *Serrate* antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the *Serrate* antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2448-2451; Renneisen et al., 1990, J. Biol. Chem. 265:16337-16342).

5.12. THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be

achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (*see* Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*)

In yet another embodiment, the Therapeutic can b delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performanc , Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); *see also* Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (*see*, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controll d release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

In specific embodiments directed to treatment or prevention of particular disorders, preferably the following forms of administration are used:

Disorder	Preferred Forms of Administration
Cervical cancer	Topical
Gastrointestinal cancer	Oral; intravenous
Lung cancer	Inhaled; intravenous
Leukemia	Intravenous; extracorporeal
Metastatic carcinomas	Intravenous; oral
Brain cancer	Targeted; intravenous;intrathecal
Liver cirrhosis	Oral; intravenous
Psoriasis	Topical
Keloids	Topical
Baldness	Topical
Spinal cord injury	Targeted; intravenous; intrathecal
Parkinson's disease	Targeted; intravenous; intrathecal
Motor neuron disease	Targeted; intravenous; intrathecal
Alzheimer's disease	Targeted; intravenous; intrathecal

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to

provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation

will also depend on the route of administration, and the seriousness of the dis ase or disorder, and should b decided according to the judgm nt of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

5.13. DIAGNOSTIC UTILITY

Vertebrate Serrate proteins, analogues, derivatives, and subsequences thereof, vertebrate *Serrate* nucleic acids (and sequences complementary thereto), anti-vertebrate Serrate antibodies, have uses in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting Serrate expression, or monitor the treatment thereof. In particular, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti-Serrate antibody und r conditions such that immunospecific binding can occur, and d tecting or measuring the amount of any immunospecific

binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, preferably in conjunction with binding of anti-Notch antibody can be used to detect aberrant Notch and/or Serrat localization or aberrant l vels of Notch-Serrate colocalization in a disease state. In a specific embodiment, antibody to Serrate can be used to assay in a patient tissue or serum sample for the presence of Serrate where an aberrant level of Serrate is an indication of a diseased condition. Aberrant levels of Serrate binding ability in an endogenous Notch protein, or aberrant levels of binding ability to Notch (or other Serrate ligand) in an endogenous Serrate protein may be indicative of a disorder of cell fate (*e.g.*, cancer, etc.) By "aberrant levels," is meant increased or decreased levels relative to that present, or a standard level representing that present, in an analogous sample from a portion of the body or from a subject not having the disorder.

The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

Vertebrate *Serrate* genes and related nucleic acid sequences and subsequences, including complementary sequences, and other toporythmic gene sequences, can also be used in hybridization assays. Vertebrate *Serrate* nucleic acid sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in Serrate expression and/or activity as described *supra*. In particular, such a hybridization assay is carri d out by a method comprising

contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to *Serrate* DNA or RNA, und r conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

Additionally, since Serrate binds to Notch, vertebrate Serrate or a binding portion thereof can be used to assay for the presence and/or amounts of Notch in a sample, *e.g.*, in screening for malignancies which exhibit increased Notch expression such as colon and cervical cancers.

6. ISOLATION AND CHARACTERIZATION OF A MOUSE *SERRATE* HOMOLOG

A mouse Serrate homolog, termed M-*Serrate*-1, was isolated as follows:

Mouse *Serrate-1* gene

Tissue origin: 10.5-day mouse embryonic RNA

Isolation method:

a) random primed cDNA against above RNA

b) PCR of above cDNA using

PCR primer 1: CGI(C/T)TTTGC(C/T)TIAA(A/G)(G/C)AITA(C/T)CA (SEQ ID NO: 9) {encoding RLCCK(H/E)YQ (SEQ ID NO:10)}:

PCR primer 2: TCIATGCAIGTICCICC(A/G)TT (SEQ ID NO:11) {encoding NGGTCID (SEQ ID NO:12)}

Amplification conditions: 50 ng cDNA, 1 μg each primer, 0.2 mM dNTP's, 1.8 U Taq (Perkin-Elmer) in 50 μl of supplied buffer, 40 cycles of: 94°C/30 sec, 45°C/2 min, 72°C/1 min extended by 2 sec each cycle.

Yielded a 1.8 kb fragment which was sequenced at both ends and identified as corresponding to *C-Serrate-1*

Partial DNA sequence of *M-Serrate-1*:

From 5' end:

GTCCCGCGTCACTGCCGGGGGACCCTGCAGCTTCGGCTCAGGGTCTACGCCTGTCATCGGG GGTAACACCTTCAATCTCAAGGCCAGCCGTGGCAACGACCGTAATCGCATCGTACTGCCTT TCAGTTTCACCTGGCCGAGGTCCTACACTTTGCTGGTGGAG (SEQ ID NO:13)

Protein translation of above:
SRVTAGGPCSFGSGSTPVIGGNTFNLKASRGNDRNRIVLPFSFTWPRSYTLLVE (SEQ ID NO:14) (corresponds to amino-terminal sequence upstr am of the DSL domain)

From 3′ end (but coding strand)
TCTTCTAACGTCTGTGGTCCCCATGGCAAGTGCAAGAGCCAGTCGGCAGGCAAATTCACCT GTGACTGTAACAAAGGCTTCACCGGCACCTACTGCCATGAAAATATCAACGACTGCGAGAG CAACCCCTGTAAA (SEQ ID NO:15)
Protein translation of above:
SSNVCGPHGKCKSQSAGKFTCDCNKGFTGTYCHENINDCESNPCK (SEQ ID NO:16) (within tandemly arranged EGF-like repeats)

Expression pattern: The expression pattern was determined to be the same as that observed for *C-Serrate-1* (chicken Serrate) (see Section 11 *infra*), including expression in the developing central nervous system, peripheral nervous system, limb, kidney, lens, and vascular system.

7. ISOLATION AND CHARACTERIZATION OF A *XENOPUS SERRATE* HOMOLOG

A *Xenopus* Serrate homolog, termed *Xenopus Serrate*-1 was isolated as follows:

Xenopus *Serrate-1* gene
Tissue origin: neurula-stage embryonic RNA
Isolation method:
a) random primed cDNA against above RNA
b) PCR using:
Primer 1: CGI(C/T)TTTGC(C/T)TIAA(A/G)(G/C)AITA(C/T)CA (SEQ ID NO:9) {encoding RLCCK(H/E)YQ (SEQ ID NO:10)}:
PCR primer 2: TCIATGCAIGTICCICC(A/G)TT (SEQ ID NO:11) {encoding NGGTCID (SEQ ID NO:12)}
Amplification conditions: 50 ng cDNA, 1 μg each primer, 0.2 mM dNTP's, 1.8 U Taq (Perkin-Elmer) in 50 μl of supplied buffer. 40 cycles of: 94°C/30 sec, 45°C/2 min, 72°C/1 min xtended by 2 s c each cycle.

Yielded a ~700 bp fragment which was partially sequenced to confirm its relationship to *C-Serrate-1*.

8. ISOLATION AND CHARACTERIZATION OF A CHICK *SERRATE* HOMOLOG

In the example herein, we report the cloning and sequence of a chick *Serrate* homolog, C-*Serrate*, and of fragments of two chick Notch homologs, C-*Notch*-1 and C-*Notch*-2, together with their expression patterns during early embryogenesis. The patterns of transcription of C-*Serrate* overlaps with that of C-*Notch*-1 in many regions of the embryo, suggesting that C-Notch-1, like Notch in *Drosophila*, is a receptor for Serrate. In particular, Notch and Serrate are expressed in the neurogenic regions of the developing central and peripheral nervous system.

Our data show that Serrate, a known ligand of Notch, has been conserved from arthropods to chordates. The overlapping expression patterns suggest conservation of its functional relationship with Notch and imply that development of the chick and in particular of its central nervous system involves the interaction of C-Notch-1 with Serrate at several specific locations.

Materials and Methods

Embryos

White Leghorn chicken eggs were obtained from University Park Farm and incubated at 38°C. Embryos were staged according to Hamburger and Hamilton (1951, J. Exp. Zool. 88:49-92).

Cloning of chicken homologs of Notch

Approximately 1000 base pair PCR fragments of the chicken *Notch 1* and *Notch 2* genes were amplified from otic explant RNA (see below) using degenerate primers and PCR conditions as outlined in Lardelli and Lendahl (1993, Exp. Cell R s. 204:364-372). The PCR fragment was subclon d into Bluescript KS-, sequenced and used as a template for making a

DIG antisens RNA probe (RNA Transcription Kit, Stratagene; DIG RNA labelling mix, Boehringer Mannheim).

Cloning of a chicken homologue of Drosophila Serrate

Otic explants were dissected from embryos of stages 8 to 13. Each otic explant consisted of the two otic cups, a short section of intervening hindbrain and pharynx and the associated head ectoderm and mesenchyme. RNA was extracted using a modification of standard protocols (Sambrook et al., 1989, *in* Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) and polyA$^+$ mRNA was isolated from total RNA using the PolyATtract mRNA Isolation System (Promega). First strand cDNA was synthesized using the SuperScript Preamplification System (Gibco).

PCR and degenerate primers were used to amplify a fragment of a chicken gene homologous to the *Drosophila* gene *Serrate* from the otic explant cDNA. The primers were designed to recognize peptide motifs found in both the fly Delta and Serrate proteins:

1) primer 1, 5-CGI(T/C)TITGC(T/C)TIAA(G/A)(G/C)AITA(C/T)CA-3' (SEQ ID NO:17), corresponds to the motif RLCLK(E/H)YQ (SEQ ID NO:18) located at the amino-terminus of the fly Delta and Serrate proteins.

2) primer 2, 5'-TCIATGCAIGTICCICC(A/G)TT-3'(SEQ ID NO:11), corresponds to the motif NGGTCID (SEQ ID NO:12) found in several of the EGF-like repeats. The PCR conditions were as follows: 35 cycles of 94°C for 1 minute, 45°C for 1.5 minutes and 72°C for 2 minutes; followed by a final extension step f 72°C for 10 minutes. A PCR product of approximately 900 base pairs in length was purified, subcloned into Bluescript KS- (Stratagene) and its DNA sequence partially determined to confirm that it was a likely Serrate homolog. It was then used to recover larger cDNA clones by screening two cDNA libraries:

1) a stage 8-13 otic explant random primed cDNA library

2) a stage 17 chick spinal cord oligo dT primed cDNA library Overlapping cDNAs wer isolated, and two (termed 9 and 3A.1) that together cover almost the entire coding region of the gene were subcloned into Bluescript KS-. DNA sequence was determined from nested deletion series generated using the double-stranded Nested Deletion Kit (Pharmacia) and Sanger dideoxy chain termination method with the Sequenase enzyme (US Biochemical Corporation). Sequences were aligned and analyzed using Geneworks 2.3 and Intelligenetics. Homology searches were done using the program Sharq.

To obtain the most 5′ end of the open reading frame, a number of other PCR based strategies were used including the screening of a number of other libraries (CDNA and genomic) using the method of Lardelli et al. (1994, Mechanisms of Development 46:123-136).

In situ hybridization

Patterns of gene transcription were determined by *in situ* hybridization using DIG-labeled RNA probes and:

1) a high-stringency wholemount *in situ* hybridization protocol, and

2) *in situ* hybridization on cryostat sections based on th protocol of Strähle et al. (1994, Trends in Genet. 10:7).

Results

To obtain insight into the likely role of chick Serrate in the vertebrate embryo, we examined its expression in relation to that of chick Notch, since functional coupling of Notch and Serrate occurs in *Drosophila*. Two chick *Notch* homologs were obtained as described below.

C-*Notch*-1 and C-*Notch*-2 are apparent counterparts of the rodent *Notch*-1 and *Notch*-2 genes, respectively

We searched for Notch homologs in the chick by PCR, using cDNA pr pared from two-day chick embryos and degenerate primers based on conserved r gions common to the known rodent *Notch* homologs. in this way, we obtained fragments, each

approximately 1000 nucleotides long, of two distinct genes, which we have called C-*Notch*-1 and C-*Notch*-2. The fragments ext nd from the third Notch/lin12 repeat up to and including the last five or so EGF-like repeats. EGF-like repeats are present in a large number of proteins, most of which are otherwise unrelated to Notch. The three Notch/lin12 repeats, however, are peculiar to the Notch family of genes and are found in all its known members. C-*Notch*-1 shows the highest degree of amino-acid identity with rodent *Notch*1 (Weinmaster et al., 1991, Development 113:199-205), and is expressed in broadly similar domains to rodent *Notch*1 (see below). Of the rodent Notch genes, C-*Notch*-2 appears most similar to *Notch*2 (Weinmaster et al., 1992, Development 116:931-941).

We examined the expression patterns of C-*Notch*-1 in early embryos by *in situ* hybridization. C-*Notch*-1 was expressed in the 1- to 2-day chick embryo in many well-defined domains, including the neural tube, the presomitic mesoderm, the nephrogenic mesoderm (the prospective mesonephros), the nasal placode, the otic placode/vesicle, the lens placode, the epibranchial placodes, the endothelial lining of the vascular system, in the heart, and the apical ectodermal ridges (AER) of the limb buds. These sites match the reported sites of Notch1 expression in rodents at equivalent stages (Table II). Taking the sequence data together with the expression data, we conclude that C-*Notch*-1 is either the chick ortholog of rodent *Notch*1, or a very close relative of it.

Table II

COMPARISON OF DOMAINS OF RODENT-*NOTCH*1 AND CHICK *NOTCH*-1 EXPRESSION THROUGHOUT EMBRYOGENESIS

Body Region	R-*Notch*1*	C-*Notch*1
primitive streak	+	+
Hensen's node	-	-
neural tube	+	+

r tina	+	+
lens	+	+
otic placode/vesicle	+	+
epibranchial placodes	+	+
nasal placode	+	+
dorsal root ganglia	+	+
presomitic mesoderm	+	+
somites	+	+
notochord	?	+
mesonephric kidney	+	+
metanephric kidney	+	+
blood vessels	+	+
heart	+	+
whisker follicles	+	N/A
thymus	+	?
toothbuds	+	N/A
salivary gland	+	?
limb bud (AER)	?	+

* from Weinmaster et al., 1991, Development 113:199-205; Franco del Amo et al., 1992, Development 115:737-744; Reaume et al., 1992, Dev. Biol. 154:377-387; Kopan and Weintraub, 1993, J. Cell. Biol. 121:631-641; Lardelli et al., 1994, Mech. of Dev. 46:123-126.

C-*Serrate* is a homolog of Drosophila *Serrate*, and codes f r a candidate ligand for a receptor belonging to the Notch family

In *Drosophila*, two ligands for Notch are known, encoded by the two related genes *Delta* and *Serrate*. The amino-acid sequences corresponding to these genes are homologous at their 5' ends, including a region, the DSL motif, which is necessary and sufficient for in vitro binding to Notch. To isolate a fragment of a chicken homolog of *Serrate*, we used PCR and degenerate primers designed to recognize sequences on ither side of the DSL motif (s Materials and methods). A 900 bas pair PCR fragment was

recovered and used to screen a library, allowing us to isolate ov rlapping cDNA clones. The DNA sequence of the cDNA clones revealed an alm st complete single open reading frame of 3582 nucleotides, lacking only a few 5' bases. Comparison with the amino acid sequences of *Drosophila* Delta and Serrate suggests that we are missing only the portion of the coding sequence that encodes part of the signal sequence of the chick Serrate protein.

Translation of the nucleotide sequence (SEQ ID NO:5) (Fig. 3) predicts a protein of 1230 amino acids (SEQ ID NO:6) (Fig. 4). A hydropathy plot reveals a single hydrophobic region characteristic of a transmembrane domain (Kyte and Doolittle, 1982, J. Mol. Biol. 157:105-132). In addition, the protein has sixteen EGF-like repeats organiz d in a tandem array in its extracellular domain. Comparison of the chick sequence with sequences of *D. melanogaster* Delta and Serrate suggests that the clones encode a chicken homolog of Serrate (Fig. 5; Fig. 6). Whereas *Drosophila* Serrate contains 14 EGF-like repeats with large insertions in repeats 4, 6 and 10, the chicken homolog has an extra two EGF-like repeats and only one small insertion of 16 amino acids in the 10th repeat. Both proteins have a second cysteine-rich region between the EGF-like repeats and the transmembrane domain; the spacing of the cysteines in this region is almost identical in the two proteins (compare $CX_2CXCX_6CX_4CX_{15}CX_5CX_7CX_4CX_5C$ in *Drosophila* Serrate with $CX_2CXCX_6CX_4CX_9CX_5CX_7CX_4CX_5C$ in C-Serrate). The intracellular domain of C-Serrate bears no significant homology to the intracellular domains of either *Drosophila* Delta or Serrate.

C-*Serrate* is expressed in the central nervous system, cranial placodes, nephric mesoderm, vascular system, and limb bud mesenchyme

In situ hybridization was performed to examine the expression of C-*Serrate* in whole-mount preparations during early mbryogenesis, from stage 4 to stage 21, at intervals

of roughly 12 hours. Lat r stages were studied by *in situ* hybridization on cryosections.

The main sites of early expr ssion of C-*Serrate*, as seen in whole mounts, can be grouped under five headings: central nervous system, cranial placodes, nephric mesoderm, vascular system, and limb bud mesenchyme.

Central nervous system

The first detectable expression of C-*Serrate* was seen in the central nervous system at stage 6 (O somites/24 hrs), within the posterior portion of the neural plate. By stage 10 (9-11 somites/35.5 hrs), a strong stripe of expression was seen in the prospective diencephalon. Additional faint staining was seen in the hindbrain and in the prospective spinal cord.

At stage 13, there were several patches of expression in the neural tube. In the diencephalon, there was a strong triangular stripe of expression that appeared to correspond to neuromere D2. There were two patches (one on either side of the midline) on the floor of the anterior mesencephalon as well as diffuse staining in the dorsal mesencephalon. In the hindbrain and rostral spinal cord, there were two longitudinal stripes of expression on either side of the midline: one along the dorsal edge of the neural tube and a second more ventral one, adjacent to the floor plate. Both were located within the domain of (rat) *Notch 1* expression. The anterior limit of the ventral stripe was at the midbrain/hindbrain boundary. The dorsal stripe was continuous with the expression in the dorsal mesencephalon. In the anterior spinal cord, expression was more spotty, the stripes being replaced by isolated scattered cells expressing *C-Serrate*.

At stage 17 (58 hrs), expression in the diencephalon and midbrain was unchanged. In the hindbrain and spinal cord, there were an additional two longitudinal strip s: one midway along the dorsoventral axis and a second wid r more ventral stripe; the anterior limits of th se

stripes coincided with the anterior border of rhombomere 2. All four longitudinal stripes in the hindbrain continued into the spinal cord of the embryo; decreasing towards its posterior end. These stripes of expression were maintained at least up to and including stage 31 (E7). By stage 21 (84 hrs), additional expression was seen in the cerebral hemispheres and strong expression in a salt and pepper distribution of cells in the optic tectum.

Cranial placodes

It is striking that C-*Serrate* is expressed in all the cranial placodes - the lens placode, the nasal placode, the otic placode/vesicle and the epibranchial placodes, as well as a patch of cranial ectoderm anterior to the otic placode that may correspond to the trigeminal placode (which is not well-defined morphologically).

In the lens placode, expression was already seen at stage 11, rapidly became very strong, and persisted at least to stage 21. Expression was weaker in the nasal placode and was only detected from stage 13. Again, expression was maintained at least until stage 21.

Likewise for the otic placode, expression began to be visible at stage 10 and was strong by early stage 11 (12-14 somites, 42.5 hours). Curiously, there was a "hole" in the otic expression domain - an anteroventral region of the placode in which the gene was not expressed. Subsequently, as the placode invaginates to form an otic vesicle, the strongest expression was seen at the anterolateral and posteromedial poles. Later still, as the otic vesicle becomes transformed into the membranous labyrinth of the inner ear, C-Serrate expression became restricted to the sensory patches.

The epibranchial expression was seen at stage 13/14 as strong staining in the ectoderm around the dorsal margins of the first and second branchial clefts. It was accompanied by expression of the gene in the deep part of the lining of

the clefts and in the endodermal lining of the branchial pouches, where the two epithelia abut one another.

Lastly, a large and strong but transient patch of expression was seen in the cranial ectoderm just anterior and ventral to the ear rudiment at stage 11. From its location, we suspect this to be, or to include, the region of the trigeminal placode.

Nephric mesoderm

Expression was detectable in the cells of the intermediate mesoderm from stage 10 and in older embryos (stage 17 to 21) in the developing mesonephric tubules.

Limb buds

C-Serrate mRNA was localized to a patch of mesenchyme at the distal end of the developing limb bud. This may suggest a role in limb growth.

Other sites

Expression was also seen in the tail bud, allantoic stalk, and possibly other tissues at late stages.

All major sites of C-*Serrate* expression lie within domains of C-*Notch*-1 expression

The conservation of the DSL domain and adjacent N-terminal region in C-Serrate suggests that it functions as a ligand for a receptor belonging to the Notch family. We thus expected to find sites where C-*Serrate* expression is accompanied by expression of a *Notch* gene. At such sites, overlapping or contiguous expression of the two genes can be taken as an indication that cells are communicating by Serrate-Notch signalling. We have compared the expression pattern of C-*Serrate*, as shown by *in situ* hybridization, with that of C-*Notch*-1, to discover what overlaps in fact occur, over a range of stages up to 8 days of incubation (E8). All th obs rved sites of C-*Serrate* expression indeed lay within,

or very closely adjacent to, domains of expression of C-*Notch*-1 (Table III).

Table III

COMPARISON OF C-NOTCH-1 AND C-SERRATE EXPRESSION AT STAGE 17a

Body region	C-*Notch*-1	C-*Serrate*
brain and spinal cord	++ (almost everywhere)	++ (specific regions)
retina	++	-
lens	+	++
otic placode/vesicle	++	++
epibranchial placodes	++	++
nasal placode	++	++
dorsal root ganglia	+	-
branchial mesenchyme	-	-
branchial ectoderm	+	++ (furrows)
branchial endoderm	+	++ (tips of pouches)
presomitic mesoderm	++	-
somites	++	-
notochord	++	-
mesonephric kidney	++	++
metanephric kidney	++	++
blood vessels	++	++
heart	+	++
limb bud (stage 21)	++ (AER)	++ (distal mesenchyme)

a Hamburger and Hamilton, 1951, J. Exp. Zool. 88:49-92.

Because of the importance of Notch and its partners in insect neurogenesis, it was of particular interest to us to see whether the homologous genes are involved in the development of the vertebrate CNS. C-Serrate is expressed in the CNS, and its pattern of expression shows a remarkable relationship to that of the Notch homologs.

We analyzed transverse sections through the spinal cord of a six day chicken embryo hybridized with *C-Notch-1*

and *C-S rrat* antis nse RNA probes. *C-Notch-1* was expressed throughout th luminal region as described previously; within this r gion, there w r two small patches in which *Serrate* was strongly expressed.

Discussion

In *Drosophila* development, cell-cell signalling via the product of the *Notch* gene plays a cardinal role in th final cell-fate decisions that specify the detailed pattern of differentiated cell types. This signalling pathway, in which the Notch protein has been identified as a transmembrane receptor, is best known for its role in neurogenesis: loss-of-function mutations in *Notch* or any of a set of other genes required for signal transmission via Notch alter cell fates in the neuroectoderm, causing cells that should have remained epidermal to become neural instead. Notch-dependent signalling is, however, as important in non-neural as in neural tissues. It regulates choices of mode of differentiation in oogenesis, in myogenesis, in formation of the Malpighian tubules and in the gut, for example, as well as in development of the retina, the peripheral sensilla, and the central nervous system. In most of these cases the signal delivered via Notch appears to mediate lateral inhibition, a type of interaction by which a cell that becomes committed to differentiate in a particular way - for example, as a neuroblast - inhibits its immediate neighbors from doing likewise. This forces adjacent cells to behave in contrasting ways, creating a fine-grained pattern of different cell types.

There are, however, good reasons to believe that this is not the only function of signals delivered via Notch. Two direct ligands of Notch have been identified. These are the products of the Delta and Serrate genes. Both of them, like *Notch* itself, code for transmembrane proteins with tandem arrays of EGF-like repeats in their extracellular domain. Both the D lta and the Serrate protein have been shown to bind to Notch in a cell adhesion assay, and th y

share a large region of homology at their amino-termini including a motif that is necessary and sufficient for interaction with Notch in vitro, the so-called EBD or DSL domain. Yet despite these biochemical similarities, they seem to have quite different developmental functions. Although Serrate is expressed in many sites in the fly, it is apparently required only in the humeral, wing and halteres disks. When Serrate function is lost by mutation, these structures fail to grow. Studies on the wing disc have indicated that it is specifically the wing margin that depends on Serrate; when Serrate is lacking, this critical signaling region and growth centre fails to form, and when *Serrate* is expressed ectopically under a GAL4-UAS promoter in the ventral part of the wing disc, ectopic wing margin tissue is induced, leading to ectopic outgrowths. Notch appears to be the receptor for Serrate at the wing margin, since some mutant alleles of Notch cause similar disturbances of wing margin development and allele-specific interactions are seen in the effects of the two genes.

Here we describe the identification and full length sequence of a homolog of the *Drosophila* gene *Serrate*, and identification and partial sequence of chick homologs of rat/mouse *Notch1* and *Notch2*.

Within the chick Serrate cDNA there is a single open reading frame predicted to encode a large transmembrane protein with 16 EGF repeats in its extracellular domain. It has a well conserved DSL motif suggesting that it would interact directly with Notch. The intracellular domain of chick Serrate exhibits no homology to anything in the current databases including the intracellular domains of *Drosophila* Delta and Serrate. It should he pointed out however that the intracellular domains of chick and human Serrate (see Section 12) are almost identical.

The spatial distributions of *C-Notch-1* and *C-Serrate* were investigated during early embryogenesis by *in situ* hybridization. *C-Notch-1* and C-Serrate exhibit dynamic and complex patterns of expression including several regions

in which th y are coexpressed (CNS, ear, branchial region, lens, h art, nasal placodes and m sonephros). The overlapping xpression together with the finding that C-Serrate has a well conserved Notch binding domain sugg sts that this receptor/ligand interaction has been conserved from *Drosophila* through to vertebrates.

In *Drosophila*, the Notch receptor is quite widely distributed and its ligands are found in overlapping but more restricted domains. In the chick a similar situation is observed.

Fly Notch is necessary for many steps in the development of *Drosophila;* its role in lateral inhibition especially in the development of the central nervous system and peripheral sense organs being the best studied examples. However, Notch is a multifunctional receptor and can interact with different signalling molecules (including Delta and Serrate) and in developmental processes that do not easily fit within the framework of lateral inhibition. While available evidence implicates Delta as the signalling molecule in lateral inhibition there is no data to suggest that Serrate participates in lateral inhibition. Rather, Serrate appears to be necessary for development of the dorsal imaginal discs of the larva; that is, the humeral, haltere and wing discs. In the latter, the best studied of these processes, Serrate and Notch are important for the development of the dorsoventral wing margin, a structure necessary for the organization of wing development as a whole.

That C-Serrate has a significant function can be inferred from the conservation of its sequence, in particular, of its Notch-binding domain. The expression patterns reported for C-Serrate in this paper provide the following information. First, since the *Serrate* gene is expressed in or next to sites where *C-Notch*-1 is expressed (possibly in conjunction with other *Notch* homologs), it is highly probable that C-Serrate exerts its action by binding to C-Notch-1 (or to another chick Notch homolog with a

similar expression pattern). Second, the expression in the developing kidn y, the vascular system and the limb buds might r flect an involvem nt in inductive signalling between mesoderm and ectoderm, which plays an important part in the development of all these organs. In the limb buds, for example, C-Serrate is expressed in the distal mesoderm, and C-Notch-1 is expressed in the overlying apical ectodermal ridge, whose maintenance is known to depend on a signal from the mesoderm below. In the cranial placodes, a similar role is possible, but the evidence for inductive signalling is weaker, and C-Serrate may equally be involved in communications between cells within the placodal epithelium, for example, in regulating the specialized modes of differentiation of the placodal calls.

What might C-Serrate's function be within the curiously restricted domains of its expression in the CNS? One possibility is that it is involved in regulating the production of oligodendrocytes, which have likewise been reported to originate from narrow bands of tissue extending along the cranio-caudal axis of the neural tube.

9. ISOLATION AND CHARACTERIZATION OF HUMAN *SERRATE* HOMOLOGS

Clones for the human *Serrate* sequence were obtained as described below.

The polymerase chain reaction (PCR) was used to amplify DNA from a human placenta cDNA library. Degenerate oligonucleotide primers used in this reaction were designed based on amino-terminal regions of high homology between *Drosophila* Serrate and *Drosophila* Delta (see Fig. 5); this high homology region includes the 5′ "DSL" domain, that is believed to code for the Notch-binding portion of Delta and Serrate. Two PCR products were isolated and used, one a 350 bp fragment, and one a 1.2 kb fragment. These PCR fragments were labeled with ^{32}P and used to screen a commercial human fetal brain cDNA library made from a 17-18 we k old fetus

(previously available from Stratagene), in which the cDNAs were inserted into the *Eco*RI site of a λ-Zap vector.

The 1.2 kb fragment hybridized to a single clone out of the 10^6 clones screened. We rescued this fragment from the λ DNA by converting the isolated phage λ clone to a plasmid via the manufacturer's instructions, yielding the *Serrate*-homologous cDNA as an insert in the *Eco*RI site of the vector Bluescript KS- (Stratagene). This plasmid was named "pBS39" and the gene corresponding to this cDNA clone was called *Human Serrate-1* (also known as *Human Jagged-1* ("*HJ1*")). The isolated cDNA was 6464 nucleotides long and contained a complete open reading frame as well as 5′ and 3′ untranslated regions (Fig. 1). Sequencing was carried out using the Sequenase® sequencing system (U.S. Biochemical Corp.) on 5 and 6% Sequagel acrylamide sequencing gels.

The 350 bp fragment hybridized with two clones, containing cDNA inserts of approximately 1.1 and 3.1 kb in length; the plasmid constructs containing these inserts were named pBS14 and pBS15, respectively. Each clone was isolated, its respective insert rescued from the λ cDNA, and sequenced as above. The nucleotide sequence of the pBS14 insert was identical to a 1.1 kb stretch of sequence contained internally within the pBS15 cDNA insert and therefore, this clone was not characterized further. The sequence of the 3.1 kb pBS15 insert encoded a single open reading frame which spanned all but the 5′ 20 nucleotides of the insert. The methionine located at the amino terminal residue of this predicted open reading was homologous to the start methionine encoded by the *Human Serrate-1* (*HJ1*) cDNA clone in pBS39. The gene encoding the cDNA insert of pBS15 was named *Human Serrate-2* and is also known as *Human Jagged-2* ("*HJ2*").

The pBS15 (*HJ2*) 3.1 kb insert was then labeled with ^{32}P and used to screen another human fetal brain library (from Clontech), in which cDNA generated from a 25-26 week-old fetus was cloned into the *Eco*RI site of λgt11. This screen identified three potential positive clones. To isolate the

cDNAs, λgt11 DNA was prepared from a liquid lysate and purified over a DEAE column. The purified DNA was then cut with *Eco*RI and the cDNA inserts w re isolated and subcloned into the *Eco*RI site of Bluescript KS-. The bluescript constructs containing these cDNAs were named pBS3-15, pBS3-2, and pBS3-20. Two of these cDNA clones, pBS3-2 and pBS3-20, contained sequences that partially overlapped with pBS15 and were further characterized. pBS3-2 had a 3.2 kb insert extending from nucleotide 1210 of the pBS15 cDNA insert to just after the polyadenylation signal. The 2.6 kb insert of pBS3-20, was restriction mapped and partially sequenced to determine its 3′ and 5′ ends. This analysis indicated that the PBS3-20 insert had a nucleic acid sequence that was fully contained within the pBS3-2 cDNA insert and therefore, the pBS3-20 insert was not characterized further. The insert of pBS3-15 was determined to be a Bluescript vector fragment contaminant.

Alignment of the deduced amino acid sequence (SEQ ID NO:4) of the "complete" *Human Serrate-2 (HJ2)* cDNA (SEQ ID NO:3) generated on the computer with the deduced amino acid sequence of Human Serrate-1 (HJ1) from pBS39 (SEQ ID NO:2) revealed a gap of about 120 bases, leading to a frameshift, in the region encoded by the pBS15 *(HJ2)* insert, between the putative signal sequence and the beginning of the DSL domain (Fig. 2). The nucleotides missing in the gap of the pBS15 insert would be located between nucleotides 240 and 241 of SEQ ID NO:3. This missing region probably resulted from a cloning artifact in the construction of the Stratagene library.

Attempts to clone the 5′ end of *HJ2* using anchored PCR, RACE, and Takara extended PCR techniques were unsuccessful. However, three human genomic clones potentially containing the 5′ end of *HJ2* were obtained from the screening of a human genomic cosmid library in which 30 kb fragments were cloned into a unique *Xho*1 site introduced into the *Bam*HI sit of a pWE15 vector (the unmodified vector is available from Stratagene). This cosmid library was

screen d with a PCR fragment that had been amplified from the 5′ end of pBS15 (*HJ2*) and thr e positive cosmid clones were is lated. Two different s ts of primers were used to amplify DNA corresponding to the 5′ end of pBS15 using the cosmid clones as a template, and both sets generated single bands that were subcloned, but which were determined to contain PCR artifacts. Portions of the cosmid clones are being subclon d directly without PCR, in order to obtain a portion of the cosmid clones that contains the 120 nucleotide stretch of DNA that is missing from pBS15.

The pBS39 cDNA insert, encoding the *Human Serrate-1* homolog (*HJ1*), has been sequenced and contains the complete coding sequence for the gene product. The nucleotide (SEQ ID NO:1) and protein (SEQ ID NO:2) sequences are shown in Figure 1. The nucleotide sequence of *Human Serrate-1* (*HJ1*) was translated using MacVector software (International Biotechnology Inc., New Haven, CT). The coding region consists of nucleotide numbers 371-4024 of SEQ ID NO:1. The Protean protein analysis software program from DNAStar (Madison, WI) was used to predict signal peptide and transmembrane regions (based on hydrophobicity). The signal peptide was predicted to consist of amino acids 14-29 of SEQ ID NO:2 (encoded by nucleotide numbers 410-457 of SEQ ID NO:1), whereby the amino terminus of the mature protein was predicted to start with Gly at amino acid number 30. The transmembrane domain was predicted to be amino acid numbers 1068-1089 of SEQ ID NO:2, encoded by nucleotide numbers 3572-3637 of SEQ ID NO:1. The consensus (DSL) domain, the region of homology with *Drosophila* Delta and Serrate, predicted to mediate binding with Notch (in particular, Notch ELR 11 and 12), spans amino acids 185-229 of SEQ ID NO:2, encoded by nucleotide numbers 923-1057 of SEQ ID NO:1. Epidermal growth factor-like (ELR) repeats in the amino acid sequence were identified by eye; 15 (full-l ngth) ELRs were identified and 3 partial ELRs as follows:

ELR 1: amino acid numbers 234 - 264

ELR 2: amino acid numbers 265 - 299

ELR 3: amino acid numb rs 300 - 339
ELR 4: amino acid numbers 340 - 377
ELR 5: amino acid numbers 378 - 415
ELR 6: amino acid numbers 416 - 453
ELR 7: amino acid numbers 454 - 490
ELR 8: amino acid numbers 491 - 528
ELR 9: amino acid numbers 529 - 566
Partial ELR: amino acid numbers 567 - 598
Partial ELR: amino acid numbers 599 - 632
ELR 10: amino acid numbers 633 - 670
ELR 11: amino acid numbers 671 - 708
ELR 12: amino acid numbers 709 - 747
ELR 13: amino acid numbers 748 - 785
ELR 14: amino acid numbers 786 - 823
ELR 15: amino acid numbers 824 - 862
Partial ELR: amino acid numbers 863 - 879
Partial ELR: amino acid numbers 880 - 896

The total ELR domain is thus amino acid numbers 234 - 896 (encoded by nucleotide numbers 1070 - 3058 of SEQ ID NO:1). The extracellular domain is thus predicted to be amino acid numbers 1 - 1067 of SEQ ID NO:2, encoded by nucleotide numbers 371 - 3571 of SEQ ID NO:1 (amino acid numbers 30 - 1067 in the mature protein; encoded by nucleotides number 458 - 3571 of SEQ ID NO:1). The intracellular (cytoplasmic) domain is thus predicted to be amino acid numbers 1090 - 1218 of SEQ ID NO:2, encoded by nucleotide numbers 3638 - 4024 of SEQ ID NO:1.

The expression of *HJ1* in certain human tissues was established by probing a Clontech Human Multiple Tissue Northern blot with radio-labeled pBS39. The probe hybridized to a single band of about 6.6 kb, and was expressed in all f the tissue assayed, which included, heart, brain, placenta, lung, skeletal muscle, pancreas, liver and kidney. The observation that *HJ1* was expressed in adult skeletal and heart muscle was particularly interesting, because adult muscle fibers are completely surrounded by a lamina of extracellular matrix, and it is unlikely, therefore, that the

role of HJ1 in these cells is in direct cell-cell communication.

The "compl t " (containing an internal deletion) *Human Serrate-2* (*HJ2*) cDNA nucleotide sequence (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) generated on the computer are shown in Figure 2. The nucleotide sequence translated using MacVector software (International Biotechnology Inc., New Haven, CT). The coding region consists of nucleotides number 332 - 4102 of SEQ ID NO:3. The Protean protein analysis software program from DNAStar (Madison, WI) was used to predict signal peptide and transmembrane regions (based on hydrophobicity). The transmembrane domain was predicted to be amino acid numb rs 912-933 of SEQ ID NO:4, encoded by nucleotides numbers 3065-3130 of SEQ ID NO:3. The consensus (DSL) domain, the region of homology with *Drosophila* Delta and Serrate, predicted to mediate binding with Notch (in particular, Notch ELR 11 and 12), spans amino acids 26-70 of SEQ ID NO:4, encoded by nucleotide numbers 407 - 541 of SEQ ID NO:3. Epidermal growth factor-like (ELR) repeats in the amino acid sequence were identified by eye; 15 (full-length) ELRs wer identified and 3 partial ELRs as follows:

ELR 1: amino acid numbers 75 - 105
ELR 2: amino acid numbers 106 - 140
ELR 3: amino acid numbers 141 - 180
ELR 4: amino acid numbers 181 - 218
ELR 5: amino acid numbers 219 - 256
ELR 6: amino acid numbers 257 - 294
ELR 7: amino acid numbers 295 - 331
ELR 8: amino acid numbers 332 - 369
ELR 9: amino acid numbers 370 - 407
Partial ELR: amino acid numbers 408 - 435
Partial ELR: amino acid numbers 436 - 469
ELR 10: amino acid numbers 470 - 507
ELR 11: amino acid numbers 508 - 545
ELR 12: amino acid numb rs 546 - 584
ELR 13: amino acid numbers 585 - 622

ELR 14: amino acid numbers 623 - 660
ELR 15: amino acid numbers 664 - 701
Partial ELR: amino acid numbers 702 - 718
Partial ELR: amino acid numbers 719 - 735

The total ELR domain is thus amino acid numbers 75 - 735 (encoded by nucleotides number 554 - 2536 of SEQ ID NO:3). The extracellular domain is thus predicted to be amino acid numbers 1 - 912 of SEQ ID NO:4, encoded by nucleotides number 332 - 3064 of SEQ ID NO:3. The intracellular (cytoplasmic) domain is thus predicted to be amino acid numbers 934 - 1257 of SEQ ID NO:4, encoded by nucleotide numbers 3131 - 4102 of SEQ ID NO:3.

Like *Human Serrate-1 (HJ1)*, the "complete" (with an internal deletion) *Human Serrate-2 (HJ2)* cDNA (SEQ ID NO:3) generated on the computer encodes a protein containing 16 complete and 2 interrupted EGF repeats as well as the diagnostic cryptic EGF repeat known as the DSL domain, which has been found only in putative Notch ligands. The open reading frame of the computer generated "complete" Human Serrate-2 (HJ2) is about 1400 amino acids long, approximately 182 amino acids longer than the carboxy terminus of HJ1 and the rat Serrate homologue Jagged. While there is significant homology between the complete HJ2 and HJ1 in the amino terminal portion of the protein, this homology is lost just before the putative transmembrane domain at about amino acid number 1029 of HJ1. This result is particularly interesting because the presence of a long COOH-terminal tail implies the possibility of some additional function or regulation of HJ2.

The "complete" (with an internal deletion) *Human Serrate-2 (HJ2)* cDNA (SEQ ID NO:3) sequence can be constructed by taking advantage of the unique restriction sites for *Acc*I, *Dra*III, or *Bam*HI present in the sequence overlap of pBS15 and pBS3-2, and which enzymes cleave the pBS15 insert at nucleotides 1431, 2648, and 2802, respectively.

The expression of *HJ2* in certain human tissues was established by probing a Clontech Human Multiple Tissue Northern blot with radio-labeled clone pBS15. This probe hybridized to a single band of about 5.2 kb and was expressed in heart, brain, placenta, lung, skeletal muscle, and pancreas, but was absent or nearly undetectable in liver and kidney. As in the case of *HJ1* expression discussed supra, the observation that the pBS15 insert component of HJ2 was expressed in adult skeletal and heart muscle was particularly interesting, because adult muscle fibers are completely surrounded by a lamina of extracellular matrix, and it is unlikely, therefore, that the role of HJ2 in these cells is in direct cell-cell communication.

Expression constructs are made using the isolated clone(s). The clone is excised from its vector as an *Eco*RI restriction fragment(s) and subcloned into the *Eco*RI restriction site of an expression vector. This allows for the expression of the Human Serrate protein product from the subclone in the correct reading frame. Using this methodology, expression constructs in which the *HJ1* cDNA insert of pBS39 was cloned into an expression vector for expression under the control of a cytomegalovirus promoter have been generated and HJ1 has been expressed in both 3T3 and HAKAT human keratinocyte cell lines.

10. DEPOSIT OF MICROORGANISMS

Plasmid pBS39, containing an *Eco*RI fragment encoding full-length Human Serrate-1 (HJ1), was deposited on February 28, 1995 with the American Type Culture Collection, 1201 Parklawn Drive, Rockville, Maryland 20852, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned Accession No. 97068.

Plasmid pBS15, containing a 3.1 kb *Eco*RI fragment encoding the amino terminus of Human Serrate-2 (HJ2), cloned into the *Eco*RI site of Blu script KS-, was deposited on March 5, 1996 with the American Type Culture Collection, 1201

Parklawn Drive, Rockville, Maryland 20852, under the provisions of th Budapest Treaty on the International Recognition of the D posit of Microorganisms for the Purposes of Patent Procedures, and assigned Accession No. _____.

Plasmid pBS3-2 containing an 3.2 kb *Eco*RI fragment encoding the carboxy terminus of Human Serrate-2 (HJ2), cloned into the *Eco*RI site of Bluescript KS-, was deposited on March 5, 1996 with the American Type Culture Collection, 1201 Parklawn Drive, Rockville, Maryland 20852, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned Accession No. _____.

The present invention is not to be limited in scope by the microorganisms deposited or the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ISH-HOROWICZ, DAVID
HENRIQUE, DOMINGOS MANUEL PINTO
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MYAT, ANNA MARY
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MANN, ROBERT S.
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(ii) TITLE OF INVENTION: NUCLEOTIDE AND PROTEIN SEQUENCES OF VERTEBRATE SERRATE GENES AND METHODS BASED THEREON

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Pennie & Edmonds
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: On Even Date Herewith
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6464 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 371..4027

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGAGGGGGAG CGTCTCAAAG AAGCGATCAG AATAATAAAA GGAGGCCGGG CTCTTTGCCT     180

TCTGGAACGG GCCGCTCTTG AAAGGGCTTT TGAAAAGTGG TGTTGTTTTC CAGTCGTGCA     240

TGCTCCAATC GGCGGAGTAT ATTAGAGCCG GGACGCGGCC GCAGGGGCAG CGGCGACGGC     300

AGCACCGGCG GCAGCACCAG CGCGAACAGC AGCGGCGGCG TCCCGAGTGC CCGCGGCGGC     360

GCGCGCAGCG ATG CGT TCC CCA CGG ACA CGC GGC CGG TCC GGG CGC CCC          409
          Met Arg Ser Pro Arg Thr Arg Gly Arg Ser Gly Arg Pro
            1               5                  10

CTA AGC CTC CTG CTC GCC CTG CTC TGT GCC CTG CGA GCC AAG GTG TGT        457
Leu Ser Leu Leu Leu Ala Leu Leu Cys Ala Leu Arg Ala Lys Val Cys
     15                  20                  25

GGG GCC TCG GGT CAG TTC GAG TTG GAG ATC CTG TCC ATG CAG AAC GTG        505
Gly Ala Ser Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val
 30                  35                  40                  45

AAC GGG GAG CTG CAG AAC GGG AAC TGC TGC GGC GGC GCC CGG AAC CCG        553
Asn Gly Glu Leu Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro
                 50                  55                  60

GGA GAC CGC AAG TGC ACC CGC GAC GAG TGT GAC ACA TAC TTC AAA GTG        601
Gly Asp Arg Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val
           65                  70                  75

TGC CTC AAG GAG TAT CAG TCC CGC GTC ACG GCC GGG GGG CCC TGC AGC        649
Cys Leu Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser
      80                  85                  90

TTC GGC TCA GGG TCC ACG CCT GTC ATC GGG GGC AAC ACC TTC AAC CTC        697
Phe Gly Ser Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu
 95                  100                 105

AAG GCC AGC CGC GGC AAC GAC CCG AAC CGC ATC GTG CTG CCT TTC AGT        745
Lys Ala Ser Arg Gly Asn Asp Pro Asn Arg Ile Val Leu Pro Phe Ser
110                 115                 120                 125

TTC GCC TGG CCG AGG TCC TAT ACG TTG CTT GTG GAG GCG TGG GAT TCC        793
Phe Ala Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser
                130                 135                 140

AGT AAT GAC ACC GTT CAA CCT GAC AGT ATT ATT GAA AAG GCT TCT CAC        841
Ser Asn Asp Thr Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His
           145                 150                 155

TCG GGC ATG ATC AAC CCC AGC CGG CAG TGG CAG ACG CTG AAG CAG AAC        889
Ser Gly Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn
      160                 165                 170

ACG GGC GTT GCC CAC TTT GAG TAT CAG ATC CGC GTG ACC TGT GAT GAC        937
Thr Gly Val Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp
 175                 180                 185

TAC TAC TAT GGC TTT GGC TGT AAT AAG TTC TGC CGC CCC AGA GAT GAC        985
Tyr Tyr Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp
190                 195                 200                 205

TTC TTT GGA CAC TAT GCC TGT GAC CAG AAT GGC AAC AAA ACT TGC ATG       1033
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Phe Phe Gly His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met
                    210                 215                 220

GAA GGC TGG ATG GGC CCC GAA TGT AAC AGA GCT ATT TGC CGA CAA GGC      1081
Glu Gly Trp Met Gly Pro Glu Cys Asn Arg Ala Ile Cys Arg Gln Gly
            225                 230                 235

TGC AGT CCT AAG CAT GGG TCT TGC AAA CTC CCA GGT GAC TGC AGG TGC      1129
Cys Ser Pro Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys
        240                 245                 250

CAG TAC GGC TGG CAA GGC CTG TAC TGT GAT AAG TGC ATC CCA CAC CCG      1177
Gln Tyr Gly Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro
    255                 260                 265

GGA TGC GTC CAC GGC ATC TGT AAT GAG CCC TGG CAG TGC CTC TGT GAG      1225
Gly Cys Val His Gly Ile Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu
270                 275                 280                 285

ACC AAC TGG GGC GGC CAG CTC TGT GAC AAA GAT CTC AAT TAC TGT GGG      1273
Thr Asn Trp Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly
                290                 295                 300

ACT CAT CAG CCG TGT CTC AAC GGG GGA ACT TGT AGC AAC ACA GGC CCT      1321
Thr His Gln Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn Thr Gly Pro
            305                 310                 315

GAC AAA TAT CAG TGT TCC TGC CCT GAG GGG TAT TCA GGA CCC AAC TGT      1369
Asp Lys Tyr Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys
        320                 325                 330

GAA ATT GCT GAG CAC GCC TGC CTC TCT GAT CCC TGT CAC AAC AGA GGC      1417
Glu Ile Ala Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly
    335                 340                 345

AGC TGT AAG GAG ACC TCC CTG GGC TTT GAG TGT GAG TGT TCC CCA GGC      1465.
Ser Cys Lys Glu Thr Ser Leu Gly Phe Glu Cys Glu Cys Ser Pro Gly
350                 355                 360                 365

TGG ACC GGC CCC ACA TGC TCT ACA AAC ATT GAT GAC TGT TCT CCT AAT      1513
Trp Thr Gly Pro Thr Cys Ser Thr Asn Ile Asp Asp Cys Ser Pro Asn
                370                 375                 380

AAC TGT TCC CAC GGG GGC ACC TGC CAG GAC CTG GTT AAC GGA TTT AAG      1561
Asn Cys Ser His Gly Gly Thr Cys Gln Asp Leu Val Asn Gly Phe Lys
            385                 390                 395

TGT GTG TGC CCC CCA CAG TGG ACT GGG AAA ACG TGC CAG TTA GAT GCA      1609
Cys Val Cys Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln Leu Asp Ala
        400                 405                 410

AAT GAA TGT GAG GCC AAA CCT TGT GTA AAC GCC AAA TCC TGT AAG AAT      1657
Asn Glu Cys Glu Ala Lys Pro Cys Val Asn Ala Lys Ser Cys Lys Asn
    415                 420                 425

CTC ATT GCC AGC TAC TAC TGC GAC TGT CTT CCC GGC TGG ATG GGT CAG      1705
Leu Ile Ala Ser Tyr Tyr Cys Asp Cys Leu Pro Gly Trp Met Gly Gln
430                 435                 440                 445

AAT TGT GAC ATA AAT ATT AAT GAC TGC CTT GGC CAG TGT CAG AAT GAC      1753
Asn Cys Asp Ile Asn Ile Asn Asp Cys Leu Gly Gln Cys Gln Asn Asp
                450                 455                 460

GCC TCC TGT CGG GAT TTG GTT AAT GGT TAT CGC TGT ATC TGT CCA CCT      1801
Ala Ser Cys Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile Cys Pro Pro
            465                 470                 475
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GGC TAT GCA GGC GAT CAC TGT GAG AGA GAC ATC GAT GAA TGT GCC AGC      1849
Gly Tyr Ala Gly Asp His Cys Glu Arg Asp Ile Asp Glu Cys Ala Ser
        480                 485                 490

AAC CCC TGT TTG AAT GGG GGT CAC TGT CAG AAT GAA ATC AAC AGA TTC      1897
Asn Pro Cys Leu Asn Gly Gly His Cys Gln Asn Glu Ile Asn Arg Phe
    495                 500                 505

CAG TGT CTG TGT CCC ACT GGT TTC TCT GGA AAC CTC TGT CAG CTG GAC      1945
Gln Cys Leu Cys Pro Thr Gly Phe Ser Gly Asn Leu Cys Gln Leu Asp
510                 515                 520                 525

ATC GAT TAT TGT GAG CCT AAT CCC TGC CAG AAC GGT GCC CAG TGC TAC      1993
Ile Asp Tyr Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala Gln Cys Tyr
                530                 535                 540

AAC CGT GCC AGT GAC TAT TTC TGC AAG TGC CCC GAG GAC TAT GAG GGC      2041
Asn Arg Ala Ser Asp Tyr Phe Cys Lys Cys Pro Glu Asp Tyr Glu Gly
            545                 550                 555

AAG AAC TGC TCA CAC CTG AAA GAC CAC TGC CGC ACG ACC CCC TGT GAA      2089
Lys Asn Cys Ser His Leu Lys Asp His Cys Arg Thr Thr Pro Cys Glu
        560                 565                 570

GTG ATT GAC AGC TGC ACA GTG GCC ATG GCT TCC AAC GAC ACA CCT GAA      2137
Val Ile Asp Ser Cys Thr Val Ala Met Ala Ser Asn Asp Thr Pro Glu
    575                 580                 585

GGG GTG CGG TAT ATT TCC TCC AAC GTC TGT GGT CCT CAC GGG AAG TGC      2185
Gly Val Arg Tyr Ile Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys
590                 595                 600                 605

AAG AGT CAG TCG GGA GGC AAA TTC ACC TGT GAC TGT AAC AAA GGC TTC      2233
Lys Ser Gln Ser Gly Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe
                610                 615                 620

ACG GGA ACA TAC TGC CAT GAA AAT ATT AAT GAC TGT GAG AGC AAC CCT      2281
Thr Gly Thr Tyr Cys His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro
            625                 630                 635

TGT AGA AAC GGT GGC ACT TGC ATC GAT GGT GTC AAC TCC TAC AAG TGC      2329
Cys Arg Asn Gly Gly Thr Cys Ile Asp Gly Val Asn Ser Tyr Lys Cys
        640                 645                 650

ATC TGT AGT GAC GGC TGG GAG GGG GCC TAC TGT GAA ACC AAT ATT AAT      2377
Ile Cys Ser Asp Gly Trp Glu Gly Ala Tyr Cys Glu Thr Asn Ile Asn
    655                 660                 665

GAC TGC AGC CAG AAC CCC TGC CAC AAT GGG GGC ACG TGT CGC GAC CTG      2425
Asp Cys Ser Gln Asn Pro Cys His Asn Gly Gly Thr Cys Arg Asp Leu
670                 675                 680                 685

GTC AAT GAC TTC TAC TGT GAC TGT AAA AAT GGG TGG AAA GGA AAG ACC      2473
Val Asn Asp Phe Tyr Cys Asp Cys Lys Asn Gly Trp Lys Gly Lys Thr
                690                 695                 700

TGC CAC TCA CGT GAC AGT CAG TGT GAT GAG GCC ACG TGC AAC AAC GGT      2521
Cys His Ser Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys Asn Asn Gly
            705                 710                 715

GGC ACC TGC TAT GAT GAG GGG GAT GCT TTT AAG TGC ATG TGT CCT GGC      2569
Gly Thr Cys Tyr Asp Glu Gly Asp Ala Phe Lys Cys Met Cys Pro Gly
        720                 725                 730

GGC TGG GAA GGA ACA ACC TGT AAC ATA GCC CGA AAC AGT AGC TGC CTG      2617
Gly Trp Glu Gly Thr Thr Cys Asn Ile Ala Arg Asn Ser Ser Cys Leu
    735                 740                 745
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CCC AAC CCC TGC CAT AAT GGG GGC ACA TGT GTG GTC AAC GGC GAG TCC      2665
Pro Asn Pro Cys His Asn Gly Gly Thr Cys Val Val Asn Gly Glu Ser
750                 755                 760                 765

TTT ACG TGC GTC TGC AAG GAA GGC TGG GAG GGG CCC ATC TGT GCT CAG      2713
Phe Thr Cys Val Cys Lys Glu Gly Trp Glu Gly Pro Ile Cys Ala Gln
                770                 775                 780

AAT ACC AAT GAC TGC AGC CCT CAT CCC TGT TAC AAC AGC GGC ACC TGT      2761
Asn Thr Asn Asp Cys Ser Pro His Pro Cys Tyr Asn Ser Gly Thr Cys
            785                 790                 795

GTG GAT GGA GAC AAC TGG TAC CGG TGC GAA TGT GCC CCG GGT TTT GCT      2809
Val Asp Gly Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro Gly Phe Ala
        800                 805                 810

GGG CCC GAC TGC AGA ATA AAC ATC AAT GAA TGC CAG TCT TCA CCT TGT      2857
Gly Pro Asp Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser Ser Pro Cys
    815                 820                 825

GCC TTT GGA GCG ACC TGT GTG GAT GAG ATC AAT GGC TAC CGG TGT GTC      2905
Ala Phe Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr Arg Cys Val
830                 835                 840                 845

TGC CCT CCA GGG CAC AGT GGT GCC AAG TGC CAG GAA GTT TCA GGG AGA      2953
Cys Pro Pro Gly His Ser Gly Ala Lys Cys Gln Glu Val Ser Gly Arg
                850                 855                 860

CCT TGC ATC ACC ATG GGG AGT GTG ATA CCA GAT GGG GCC AAA TGG GAT      3001
Pro Cys Ile Thr Met Gly Ser Val Ile Pro Asp Gly Ala Lys Trp Asp
            865                 870                 875

GAT GAC TGT AAT ACC TGC CAG TGC CTG AAT GGA CGG ATC GCC TGC TCA      3049
Asp Asp Cys Asn Thr Cys Gln Cys Leu Asn Gly Arg Ile Ala Cys Ser
        880                 885                 890

AAG GTC TGG TGT GGC CCT CGA CCT TGC CTG CTC CAC AAA GGG CAC AGC      3097
Lys Val Trp Cys Gly Pro Arg Pro Cys Leu Leu His Lys Gly His Ser
    895                 900                 905

GAG TGC CCC AGC GGG CAG AGC TGC ATC CCC ATC CTG GAC GAC CAG TGC      3145
Glu Cys Pro Ser Gly Gln Ser Cys Ile Pro Ile Leu Asp Asp Gln Cys
910                 915                 920                 925

TTC GTC CAC CCC TGC ACT GGT GTG GGC GAG TGT CGG TCT TCC AGT CTC      3193
Phe Val His Pro Cys Thr Gly Val Gly Glu Cys Arg Ser Ser Ser Leu
                930                 935                 940

CAG CCG GTG AAG ACA AAG TGC ACC TCT GAC TCC TAT TAC CAG GAT AAC      3241
Gln Pro Val Lys Thr Lys Cys Thr Ser Asp Ser Tyr Tyr Gln Asp Asn
            945                 950                 955

TGT GCG AAC ATC ACA TTT ACC TTT AAC AAG GAG ATG ATG TCA CCA GGT      3289
Cys Ala Asn Ile Thr Phe Thr Phe Asn Lys Glu Met Met Ser Pro Gly
        960                 965                 970

CTT ACT ACG GAG CAC ATT TGC AGT GAA TTG AGG AAT TTG AAT ATT TTG      3337
Leu Thr Thr Glu His Ile Cys Ser Glu Leu Arg Asn Leu Asn Ile Leu
    975                 980                 985

AAG AAT GTT TCC GCT GAA TAT TCA ATC TAC ATC GCT TGC GAG CCT TCC      3385
Lys Asn Val Ser Ala Glu Tyr Ser Ile Tyr Ile Ala Cys Glu Pro Ser
990                 995                 1000                1005

CCT TCA GCG AAC AAT GAA ATA CAT GTG GCC ATT TCT GCT GAA GAT ATA      3433
Pro Ser Ala Asn Asn Glu Ile His Val Ala Ile Ser Ala Glu Asp Ile
                1010                1015                1020
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CGG GAT GAT GGG AAC CCG ATC AAG GAA ATC ACT GAC AAA ATA ATC GAT      3481
Arg Asp Asp Gly Asn Pro Ile Lys Glu Ile Thr Asp Lys Ile Ile Asp
            1025                1030                1035

CTT GTT ACT AAA CGT GAT GGA AAC AGC TCG CTG ATT GCT GCC GTT GAA      3529
Leu Val Thr Lys Arg Asp Gly Asn Ser Ser Leu Ile Ala Ala Val Glu
        1040                1045                1050

GAA GTA AGA GTT CAG AGG CGG CCT CTG AAG AAC AGA ACA GAT TTC CTT      3577
Glu Val Arg Val Gln Arg Arg Pro Leu Lys Asn Arg Thr Asp Phe Leu
    1055                1060                1065

GTT CCC TTG CTG AGC TCT GTC TTA ACT GTG GCT TGG ATC TGT TGC TTG      3625
Val Pro Leu Leu Ser Ser Val Leu Thr Val Ala Trp Ile Cys Cys Leu
1070                1075                1080                1085

GTG ACG GCC TTC TAC TGG TGC CTG CGG AAG CGG CGG AAG CCG GGC AGC      3673
Val Thr Ala Phe Tyr Trp Cys Leu Arg Lys Arg Arg Lys Pro Gly Ser
                1090                1095                1100

CAC ACA CAC TCA GCC TCT GAG GAC AAC ACC ACC AAC AAC GTG CGG GAG      3721
His Thr His Ser Ala Ser Glu Asp Asn Thr Thr Asn Asn Val Arg Glu
            1105                1110                1115

CAG CTG AAC CAG ATC AAA AAC CCC ATT GAG AAA CAT GGG GCC AAC ACG      3769
Gln Leu Asn Gln Ile Lys Asn Pro Ile Glu Lys His Gly Ala Asn Thr
        1120                1125                1130

GTC CCC ATC AAG GAT TAC GAG AAC AAG AAC TCC AAA ATG TCT AAA ATA      3817
Val Pro Ile Lys Asp Tyr Glu Asn Lys Asn Ser Lys Met Ser Lys Ile
    1135                1140                1145

AGG ACA CAC AAT TCT GAA GTA GAA GAG GAC GAC ATG GAC AAA CAC CAG      3865
Arg Thr His Asn Ser Glu Val Glu Glu Asp Asp Met Asp Lys His Gln
1150                1155                1160                1165

CAG AAA GCC CGG TTT GCC AAG CAG CCG GCG TAC ACG CTG GTA GAC AGA      3913
Gln Lys Ala Arg Phe Ala Lys Gln Pro Ala Tyr Thr Leu Val Asp Arg
                1170                1175                1180

GAA GAG AAG CCC CCC AAC GGC ACG CCG ACA AAA CAC CCA AAC TGG ACA      3961
Glu Glu Lys Pro Pro Asn Gly Thr Pro Thr Lys His Pro Asn Trp Thr
            1185                1190                1195

AAC AAA CAG GAC AAC AGA GAC TTG GAA AGT GCC CAG AGC TTA AAC CGA      4009
Asn Lys Gln Asp Asn Arg Asp Leu Glu Ser Ala Gln Ser Leu Asn Arg
        1200                1205                1210

ATG GAG TAC ATC GTA TAG CAGACCGCGG GCACTGCCGC CGCTAGGTAG             4057
Met Glu Tyr Ile Val
    1215

AGTCTGAGGG CTTGTAGTTC TTTAAACTGT CGTGTCATAC TCGAGTCTGA GGCCGTTGCT    4117

GACTTAGAAT CCCTGTGTTA ATTTAGTTTG ACAAGCTGGC TTACACTGGC AATGGTAGTT    4177

CTGTGGTTGG CTGGGAAATC GAGTGGCGCA TCTCACAGCT ATGCAAAAAG CTAGTCAACA    4237

GTACCCCTGG TTGTGTGTCC CCTTGCAGCC GACACGGTCT CGGATCAGGC TCCCAGGAGC    4297

TGCCCAGCCC CCTGGTACTT TGAGCTCCCA CTTCTGCCAG ATGTCTAATG GTGATGCAGT    4357

CTTAGATCAT AGTTTTATTT ATATTTATTG ACTCTTGAGT TGTTTTTGTA TATTGGTTTT    4417

ATGATGACGT ACAAGTAGTT CTGTATTTGA AAGTGCCTTT GCAGCTCAGA ACCACAGCAA    4477

CGATCACAAA TGACTTTATT ATTTATTTTT TTTAATTGTA TTTTTGTTGT TGGGGGAGGG    4537
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GAGACTTTGA TGTCAGCAGT TGCTGGTAAA ATGAAGAATT TAAAGAAAAA ATGTCCAAAA 4597

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TTAGAATTGA AGGTTTTTGA TAGCATTGTA AGCGTATGGC TTTATTTTTT TGAACTCTTC 4777

TCATTACTTG TTGCCTATAA GCCAAAAAGG AAAGGGTGTT TTGAAAATAG TTTATTTTAA 4837

AACAATAGGA TGGGCTACAC GTACATAGGT AAATAATAGC ACCGTACTGG TTATGATGAT 4897

GAAAATAACT GGAAACTTGA AAGCTTGTGG TAATGGCAGA TAAAGATGGT TCACCTGGGA 4957

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TGCCAAATGG GCAGTTATTG TTTCAGGGAG AGAAGCTGCT CATTGGCCAA TCATTCTGGT 5197

GTGCAGTGCT CCATCGGATT CTACATGTCC AACAAGGCAT GTCTGGATGA TGCAATGTCT 5257

GTCTGACCCC CGGAATTCCG TGCAGAGACA ACATTCTAGA CAGATATACA CTTTTTATTA 5317

TTAACAAACT TTGGCCACAA CCTTTGATGT ATAAATTGCC GGATTTCCCC AGTCCTTTCA 5377

TTGTGGCTTT GGACAGGAGC AGGCTCACTT GTCTGCTTCA GGCTGCCTTT CTCTTGGGTT 5437

GCACCTCAGT TCTTACTTAT TTATTTATTT TGAGTGGAGC ATAGGGGCCT CTTCCAAAAT 5497

GGGTAGAGCT CAGGGGCTTT CTTATTGAAA TGGTCACATG ATAAAAACGG GCTGAAAAAG 5557

GAGAGTTCCA GGAGAAAAGC CCAGAAAAGG CCCCTCCTCA GAAGACAGCC TTTAAGCCTC 5617

TTGCTTACTG AAGGAAGCCC CACCTTCTAG CACTGAGGCC GGGTCTGATC TTCCAGAGGA 5677

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TGAGAGAACA GCGGGATCCT TGTTGTCCTC TAGAGACTTG AGTCTGTCAC TGACATTTTT 5797

TCAGTTCCTT TGCTCATAGA CCATACGAGG AATTAGTGAT GTGTCAGTTG AGAGTTCACA 5857

ATCTCATTGT TCATTTAATT CACTTTAAAG TTGTCAATTT CTGTGTGAGT AACCTGTAAA 5917

AGACACCTTT CCAGAAGAGT TTTGCCGTCT GTTTGAAAAA AAAATCTTTA TAAACTTTCC 5977

TAAGTATCTG GATTTGGATT CCTTATTTGG AGAGAAAATG TACCCTGTCT CCACCAAAAA 6037

TACAAAAATT AGCCAGGCTT GGTGGTGCAC ACCGGTAATC CCAGCAACTC TGGAGACTAA 6097

GGCAGGAAGA ATCGCTTGAC CCAGGAGGGT CGAGGCTACA ATGAGTTGAA ACCGCGCCAC 6157

TGCACTCCAG CCTGGGCGAC AGTGCGAGGC CCTGTCTCAA AAATAAAATA AAATAAATAA 6217

ATAAATTAGC CAGATACTGT GTGCACGCCT GCAGTCCCAG CTATTCTGGA AGCTGAGGTG 6277

GGAAGATGGT TAAGCCTGAG AGGACAAAGC TGCAGTGAGT CATGTTTGCA TCACTGCACT 6337

CCAGCCTGGG TGACAGAGCA AGACCCTGTC TAAAAAACAA AAACAGGCCG GGTGTGGTGG 6397

CTCATGCCTG CCATCCCAGT GCTTTGGGAG GCAGAGGTTG GCATAATCCC AGCGCTCTGG 6457

GAATTCC 6464

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1219 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Arg Ser Pro Arg Thr Arg Gly Arg Ser Gly Arg Pro Leu Ser Leu
 1               5                  10                  15

Leu Leu Ala Leu Leu Cys Ala Leu Arg Ala Lys Val Cys Gly Ala Ser
            20                  25                  30

Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly Glu
        35                  40                  45

Leu Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro Gly Asp Arg
    50                  55                  60

Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu Lys
65                  70                  75                  80

Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly Ser
                85                  90                  95

Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala Ser
            100                 105                 110

Arg Gly Asn Asp Pro Asn Arg Ile Val Leu Pro Phe Ser Phe Ala Trp
        115                 120                 125

Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn Asp
    130                 135                 140

Thr Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly Met
145                 150                 155                 160

Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly Val
                165                 170                 175

Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp Tyr Tyr Tyr
            180                 185                 190

Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe Gly
        195                 200                 205

His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly Trp
    210                 215                 220

Met Gly Pro Glu Cys Asn Arg Ala Ile Cys Arg Gln Gly Cys Ser Pro
225                 230                 235                 240

Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys Gln Tyr Gly
                245                 250                 255

Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro Gly Cys Val
            260                 265                 270

His Gly Ile Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu Thr Asn Trp
        275                 280                 285

Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Thr His Gln
    290                 295                 300
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Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn Thr Gly Pro Asp Lys Tyr
305                 310                 315                 320

Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys Glu Ile Ala
                325                 330                 335

Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly Ser Cys Lys
            340                 345                 350

Glu Thr Ser Leu Gly Phe Glu Cys Glu Cys Ser Pro Gly Trp Thr Gly
        355                 360                 365

Pro Thr Cys Ser Thr Asn Ile Asp Asp Cys Ser Pro Asn Asn Cys Ser
    370                 375                 380

His Gly Gly Thr Cys Gln Asp Leu Val Asn Gly Phe Lys Cys Val Cys
385                 390                 395                 400

Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln Leu Asp Ala Asn Glu Cys
                405                 410                 415

Glu Ala Lys Pro Cys Val Asn Ala Lys Ser Cys Lys Asn Leu Ile Ala
            420                 425                 430

Ser Tyr Tyr Cys Asp Cys Leu Pro Gly Trp Met Gly Gln Asn Cys Asp
        435                 440                 445

Ile Asn Ile Asn Asp Cys Leu Gly Gln Cys Gln Asn Asp Ala Ser Cys
    450                 455                 460

Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile Cys Pro Pro Gly Tyr Ala
465                 470                 475                 480

Gly Asp His Cys Glu Arg Asp Ile Asp Glu Cys Ala Ser Asn Pro Cys
                485                 490                 495

Leu Asn Gly Gly His Cys Gln Asn Glu Ile Asn Arg Phe Gln Cys Leu
            500                 505                 510

Cys Pro Thr Gly Phe Ser Gly Asn Leu Cys Gln Leu Asp Ile Asp Tyr
        515                 520                 525

Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala Gln Cys Tyr Asn Arg Ala
    530                 535                 540

Ser Asp Tyr Phe Cys Lys Cys Pro Glu Asp Tyr Glu Gly Lys Asn Cys
545                 550                 555                 560

Ser His Leu Lys Asp His Cys Arg Thr Thr Pro Cys Glu Val Ile Asp
                565                 570                 575

Ser Cys Thr Val Ala Met Ala Ser Asn Asp Thr Pro Glu Gly Val Arg
            580                 585                 590

Tyr Ile Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser Gln
        595                 600                 605

Ser Gly Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly Thr
    610                 615                 620

Tyr Cys His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Arg Asn
625                 630                 635                 640

Gly Gly Thr Cys Ile Asp Gly Val Asn Ser Tyr Lys Cys Ile Cys Ser
                645                 650                 655

Asp Gly Trp Glu Gly Ala Tyr Cys Glu Thr Asn Ile Asn Asp Cys Ser
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660 665 670

Gln Asn Pro Cys His Asn Gly Gly Thr Cys Arg Asp Leu Val Asn Asp
675 680 685

Phe Tyr Cys Asp Cys Lys Asn Gly Trp Lys Gly Lys Thr Cys His Ser
690 695 700

Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys Asn Asn Gly Gly Thr Cys
705 710 715 720

Tyr Asp Glu Gly Asp Ala Phe Lys Cys Met Cys Pro Gly Gly Trp Glu
725 730 735

Gly Thr Thr Cys Asn Ile Ala Arg Asn Ser Ser Cys Leu Pro Asn Pro
740 745 750

Cys His Asn Gly Gly Thr Cys Val Val Asn Gly Glu Ser Phe Thr Cys
755 760 765

Val Cys Lys Glu Gly Trp Glu Gly Pro Ile Cys Ala Gln Asn Thr Asn
770 775 780

Asp Cys Ser Pro His Pro Cys Tyr Asn Ser Gly Thr Cys Val Asp Gly
785 790 795 800

Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro Gly Phe Ala Gly Pro Asp
805 810 815

Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser Ser Pro Cys Ala Phe Gly
820 825 830

Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr Arg Cys Val Cys Pro Pro
835 840 845

Gly His Ser Gly Ala Lys Cys Gln Glu Val Ser Gly Arg Pro Cys Ile
850 855 860

Thr Met Gly Ser Val Ile Pro Asp Gly Ala Lys Trp Asp Asp Asp Cys
865 870 875 880

Asn Thr Cys Gln Cys Leu Asn Gly Arg Ile Ala Cys Ser Lys Val Trp
885 890 895

Cys Gly Pro Arg Pro Cys Leu Leu His Lys Gly His Ser Glu Cys Pro
900 905 910

Ser Gly Gln Ser Cys Ile Pro Ile Leu Asp Asp Gln Cys Phe Val His
915 920 925

Pro Cys Thr Gly Val Gly Glu Cys Arg Ser Ser Ser Leu Gln Pro Val
930 935 940

Lys Thr Lys Cys Thr Ser Asp Ser Tyr Tyr Gln Asp Asn Cys Ala Asn
945 950 955 960

Ile Thr Phe Thr Phe Asn Lys Glu Met Met Ser Pro Gly Leu Thr Thr
965 970 975

Glu His Ile Cys Ser Glu Leu Arg Asn Leu Asn Ile Leu Lys Asn Val
980 985 990

Ser Ala Glu Tyr Ser Ile Tyr Ile Ala Cys Glu Pro Ser Pro Ser Ala
995 1000 1005

Asn Asn Glu Ile His Val Ala Ile Ser Ala Glu Asp Ile Arg Asp Asp
1010 1015 1020

Gly Asn Pro Ile Lys Glu Ile Thr Asp Lys Ile Ile Asp Leu Val Thr
1025 1030 1035 1040

Lys Arg Asp Gly Asn Ser Ser Leu Ile Ala Ala Val Glu Glu Val Arg
1045 1050 1055

Val Gln Arg Arg Pro Leu Lys Asn Arg Thr Asp Phe Leu Val Pro Leu
1060 1065 1070

Leu Ser Ser Val Leu Thr Val Ala Trp Ile Cys Cys Leu Val Thr Ala
1075 1080 1085

Phe Tyr Trp Cys Leu Arg Lys Arg Arg Lys Pro Gly Ser His Thr His
1090 1095 1100

Ser Ala Ser Glu Asp Asn Thr Thr Asn Asn Val Arg Glu Gln Leu Asn
1105 1110 1115 1120

Gln Ile Lys Asn Pro Ile Glu Lys His Gly Ala Asn Thr Val Pro Ile
1125 1130 1135

Lys Asp Tyr Glu Asn Lys Asn Ser Lys Met Ser Lys Ile Arg Thr His
1140 1145 1150

Asn Ser Glu Val Glu Glu Asp Asp Met Asp Lys His Gln Gln Lys Ala
1155 1160 1165

Arg Phe Ala Lys Gln Pro Ala Tyr Thr Leu Val Asp Arg Glu Glu Lys
1170 1175 1180

Pro Pro Asn Gly Thr Pro Thr Lys His Pro Asn Trp Thr Asn Lys Gln
1185 1190 1195 1200

Asp Asn Arg Asp Leu Glu Ser Ala Gln Ser Leu Asn Arg Met Glu Tyr
1205 1210 1215

Ile Val

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4483 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 332..4483

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCCGGGGCC GGGCGGGCGG GTCGCGGGGG CAATGCGGGC GCAGGGCCGG GGGCGCCTTC 60

CCCGGCGGCT GCTGCTGCTG CTGGCGCTCT GGGTGCAGGC GGCGCGGCCC ATGGGCTATT 120

TCGAGCTGCA GCTGAGCGCG CTGCGGAACG TGAACGGGGA GCTGCTGAGC GGCGCCTGCT 180

GTGACGGCGA CGGCCGGACA ACGCGCGCGG GGGGCTGCGG CCACGACGAG TGCGACACCG 240

CTCCTTTACC CTCATCGTGG AGGCCTGGGA CTGGGACAAC GATACCACCC CGAATGAGGA 300

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GCTGCTGATC GAGCGAGTGT CGCATGCCGG C ATG ATC AAC CCG GAG GAC CGC                352
                                   Met Ile Asn Pro Glu Asp Arg
                                     1               5

TGG AAG AGC CTG CAC TTC AGC GGC CAC GTG GCG CAC CTG GAG CTG CAG               400
Trp Lys Ser Leu His Phe Ser Gly His Val Ala His Leu Glu Leu Gln
          10                  15                  20

ATC CGC GTG CGC TGC GAC GAG AAC TAC TAC AGC GCC ACT TGC AAC AAG               448
Ile Arg Val Arg Cys Asp Glu Asn Tyr Tyr Ser Ala Thr Cys Asn Lys
      25                  30                  35

TTC TGC CGG CCC CGC AAT GAC TTT TTC GGC CAC TAC ACC TGC GAC CAG               496
Phe Cys Arg Pro Arg Asn Asp Phe Phe Gly His Tyr Thr Cys Asp Gln
  40                  45                  50                  55

TAC GGC AAC AAG GCC TGC ATG GAC GGC TGG ATG GGC AAG GAG TGC AAG               544
Tyr Gly Asn Lys Ala Cys Met Asp Gly Trp Met Gly Lys Glu Cys Lys
                  60                  65                  70

GAA GCT GTG TGT AAA CAA GGG TGT AAT TTG CTC CAC GGG GGA TGC ACC               592
Glu Ala Val Cys Lys Gln Gly Cys Asn Leu Leu His Gly Gly Cys Thr
              75                  80                  85

GTG CCT GGG GAG TGC AGG TGC AGC TAC GGC TGG CAA GGG AGG TTC TGC               640
Val Pro Gly Glu Cys Arg Cys Ser Tyr Gly Trp Gln Gly Arg Phe Cys
          90                  95                  100

GAT GAG TGT GTC CCC TAC CCC GGC TGC GTG CAT GGC AGT TGT GTG GAG               688
Asp Glu Cys Val Pro Tyr Pro Gly Cys Val His Gly Ser Cys Val Glu
      105                 110                 115

CCC TGG CAG TGC AAC TGT GAG ACC AAC TGG GGC GGC CTG CTC TGT GAC               736
Pro Trp Gln Cys Asn Cys Glu Thr Asn Trp Gly Gly Leu Leu Cys Asp
  120                 125                 130                 135

AAA GAC CTG AAC TAC TGT GGC AGC CAC CAC CCC TGC ACC AAC GGA GGC               784
Lys Asp Leu Asn Tyr Cys Gly Ser His His Pro Cys Thr Asn Gly Gly
                  140                 145                 150

ACG TGC ATC AAC GCC GAG CCT GAC CAG TAC CGC TGC ACC TGC CCT GAC               832
Thr Cys Ile Asn Ala Glu Pro Asp Gln Tyr Arg Cys Thr Cys Pro Asp
              155                 160                 165

GGC TAC TCG GGC AGG AAC TGT GAG AAG GCT GAG CAC GCC TGC ACC TCC               880
Gly Tyr Ser Gly Arg Asn Cys Glu Lys Ala Glu His Ala Cys Thr Ser
          170                 175                 180

AAC CCG TGT GCC AAC GGG GGC TCT TGC CAT GAG GTG CCG TCC GGC TTC               928
Asn Pro Cys Ala Asn Gly Gly Ser Cys His Glu Val Pro Ser Gly Phe
      185                 190                 195

GAA TGC CAC TGC CCA TCG GGC TGG AGC GGG CCC ACC TGT GCC CTT GAC               976
Glu Cys His Cys Pro Ser Gly Trp Ser Gly Pro Thr Cys Ala Leu Asp
  200                 205                 210                 215

ATC GAT GAG TGT GCT TCG AAC CCG TGT GCG GCC GGT GGC ACC TGT GTG              1024
Ile Asp Glu Cys Ala Ser Asn Pro Cys Ala Ala Gly Gly Thr Cys Val
                  220                 225                 230

GAC CAG GTG GAC GGC TTT GAG TGC ATC TGC CCC GAG CAG TGG GTG GGG              1072
Asp Gln Val Asp Gly Phe Glu Cys Ile Cys Pro Glu Gln Trp Val Gly
              235                 240                 245

GCC ACC TGC CAG CTG GAC GCC AAT GAG TGT GAA GGG AAG CCA TGC CTT              1120
Ala Thr Cys Gln Leu Asp Ala Asn Glu Cys Glu Gly Lys Pro Cys Leu
          250                 255                 260
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AAC GCT TTT TCT TGC AAA AAC CTG ATT GGC GGC TAT TAC TGT GAT TGC      1168
Asn Ala Phe Ser Cys Lys Asn Leu Ile Gly Gly Tyr Tyr Cys Asp Cys
    265                 270                 275

ATC CCG GGC TGG AAG GGC ATC AAC TGC CAT ATC AAC GTC AAC GAC TGT      1216
Ile Pro Gly Trp Lys Gly Ile Asn Cys His Ile Asn Val Asn Asp Cys
280                 285                 290                 295

CGC GGG CAG TGT CAG CAT GGG GGC ACC TGC AAG GAC CTG GTG AAC GGG      1264
Arg Gly Gln Cys Gln His Gly Gly Thr Cys Lys Asp Leu Val Asn Gly
                300                 305                 310

TAC CAG TGT GTG TGC CCA CGG GGC TTC GGA GGC CGG CAT TGC GAG CTG      1312
Tyr Gln Cys Val Cys Pro Arg Gly Phe Gly Gly Arg His Cys Glu Leu
            315                 320                 325

GAA CGA GAC AAG TGT GCC AGC AGC CCC TGC CAC AGC GGC GGC CTC TGC      1360
Glu Arg Asp Lys Cys Ala Ser Ser Pro Cys His Ser Gly Gly Leu Cys
        330                 335                 340

GAG GAC CTG GCC GAC GGC TTC CAC TGC CAC TGC CCC CAG GGC TTC TCC      1408
Glu Asp Leu Ala Asp Gly Phe His Cys His Cys Pro Gln Gly Phe Ser
    345                 350                 355

GGG CCT CTC TGT GAG GTG GAT GTC GAC CTT TGT GAG CCA AGC CCC TGC      1456
Gly Pro Leu Cys Glu Val Asp Val Asp Leu Cys Glu Pro Ser Pro Cys
360                 365                 370                 375

CGG AAC GGC GCT CGC TGC TAT AAC CTG GAG GGT GAC TAT TAC TGC GCC      1504
Arg Asn Gly Ala Arg Cys Tyr Asn Leu Glu Gly Asp Tyr Tyr Cys Ala
                380                 385                 390

TGC CCT GAT GAC TTT GGT GGC AAG AAC TGC TCC GTG CCC CGC GAG CCG      1552
Cys Pro Asp Asp Phe Gly Gly Lys Asn Cys Ser Val Pro Arg Glu Pro
            395                 400                 405

TGC CCT GGC GGG GCC TGC AGA GTG ATC GAT GGC TGC GGG TCA GAC GCG      1600
Cys Pro Gly Gly Ala Cys Arg Val Ile Asp Gly Cys Gly Ser Asp Ala
        410                 415                 420

GGG CCT GGG ATG CCT GGC ACA GCA GCC TCC GGC GTG TGT GGC CCC CAT      1648
Gly Pro Gly Met Pro Gly Thr Ala Ala Ser Gly Val Cys Gly Pro His
    425                 430                 435

GGA CGC TGC GTC AGC CAG CCA GGG GGC AAC TTT TCC TGC ATC TGT GAC      1696
Gly Arg Cys Val Ser Gln Pro Gly Gly Asn Phe Ser Cys Ile Cys Asp
440                 445                 450                 455

AGT GGC TTT ACT GGC ACC TAC TGC CAT GAG AAC ATT GAC GAC TGC CTG      1744
Ser Gly Phe Thr Gly Thr Tyr Cys His Glu Asn Ile Asp Asp Cys Leu
                460                 465                 470

GGC CAG CCC TGC CGC AAT GGG GGC ACA TGC ATC GAT GAG GTG GAC GCC      1792
Gly Gln Pro Cys Arg Asn Gly Gly Thr Cys Ile Asp Glu Val Asp Ala
            475                 480                 485

TTC CGC TGC TTC TGC CCC AGC GGT TGG GAG GGC GAG CTC TGC GAC ACC      1840
Phe Arg Cys Phe Cys Pro Ser Gly Trp Glu Gly Glu Leu Cys Asp Thr
        490                 495                 500

AAT CCC AAC GAC TGC CTT CCC GAT CCC TGC CAC AGC CGC GGC CGC TGC      1888
Asn Pro Asn Asp Cys Leu Pro Asp Pro Cys His Ser Arg Gly Arg Cys
    505                 510                 515

TAC GAC CTG GTC AAT GAC TTC TAC TGT GCG TGC GAC GAC GGC TGG AAG      1936
Tyr Asp Leu Val Asn Asp Phe Tyr Cys Ala Cys Asp Asp Gly Trp Lys
520                 525                 530                 535
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GGC AAG ACC TGC CAC TCA CGC GAG TTC CAG TGC GAT GCC TAC ACC TGC          1984
Gly Lys Thr Cys His Ser Arg Glu Phe Gln Cys Asp Ala Tyr Thr Cys
                540                 545                 550

AGC AAC GGT GGC ACC TGC TAC GAC AGC GGC GAC ACC TTC CGC TGC GCC          2032
Ser Asn Gly Gly Thr Cys Tyr Asp Ser Gly Asp Thr Phe Arg Cys Ala
            555                 560                 565

TGC CCC CCC GGC TGG AAG GGC AGC ACC TGC GCC GTC GCC AAG AAC AGC          2080
Cys Pro Pro Gly Trp Lys Gly Ser Thr Cys Ala Val Ala Lys Asn Ser
        570                 575                 580

AGC TGC CTG CCC AAC CCC TGT GTG AAT GGT GGC ACC TGC GTG GGC AGC          2128
Ser Cys Leu Pro Asn Pro Cys Val Asn Gly Gly Thr Cys Val Gly Ser
    585                 590                 595

GGG GCC TCC TTC TCC TGC ATC TGC CGG GAC GGC TGG GAG GGT CGT ACT          2176
Gly Ala Ser Phe Ser Cys Ile Cys Arg Asp Gly Trp Glu Gly Arg Thr
600                 605                 610                 615

TGC ACT CAC AAT ACC AAC GAC TGC AAC CCT CTG CCT TGC TAC AAT GGT          2224
Cys Thr His Asn Thr Asn Asp Cys Asn Pro Leu Pro Cys Tyr Asn Gly
                620                 625                 630

GGC ATC TGT GTT GAC GGC GTC AAC TGG TTC CGC TGC GAG TGT GCA CCT          2272
Gly Ile Cys Val Asp Gly Val Asn Trp Phe Arg Cys Glu Cys Ala Pro
            635                 640                 645

GGC TTC GCG GGG CCT GAC TGC CGC ATC AAC ATC GAC GAG TGC CAG TCC          2320
Gly Phe Ala Gly Pro Asp Cys Arg Ile Asn Ile Asp Glu Cys Gln Ser
        650                 655                 660

TCG CCC TGT GCC TAC GGG GCC ACG TGT GTG GAT GAG ATC AAC GGG TAT          2368
Ser Pro Cys Ala Tyr Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr
    665                 670                 675

CGC TGT AGC TGC CCA CCC GGC CGA GCC GGC CCC CGG TGC CAG GAA GTG          2416
Arg Cys Ser Cys Pro Pro Gly Arg Ala Gly Pro Arg Cys Gln Glu Val
680                 685                 690                 695

ATC GGG TTC GGG AGA TCC TGC TGG TCC CGG GGC ACT CCG TTC CCA CAC          2464
Ile Gly Phe Gly Arg Ser Cys Trp Ser Arg Gly Thr Pro Phe Pro His
                700                 705                 710

GGA AGC TCC TGG GTG GAA GAC TGC AAC AGC TGC CGC TGC CTG GAT GGC          2512
Gly Ser Ser Trp Val Glu Asp Cys Asn Ser Cys Arg Cys Leu Asp Gly
            715                 720                 725

CGC CGT GAC TGC AGC AAG GTG TGG TGC GGA TGG AAG CCT TGT CTG CTG          2560
Arg Arg Asp Cys Ser Lys Val Trp Cys Gly Trp Lys Pro Cys Leu Leu
        730                 735                 740

GCC GGC CAG CCC GAG GCC CTG AGC GCC CAG TGC CCA CTG GGG CAA AGG          2608
Ala Gly Gln Pro Glu Ala Leu Ser Ala Gln Cys Pro Leu Gly Gln Arg
    745                 750                 755

TGC CTG GAG AAG GCC CCA GGC CAG TGT CTG CGA CCA CCC TGT GAG GCC          2656
Cys Leu Glu Lys Ala Pro Gly Gln Cys Leu Arg Pro Pro Cys Glu Ala
760                 765                 770                 775

TGG GGG GAG TGC GGC GCA GAA GAG CCA CCG AGC ACC CCC TGC CTG CCA          2704
Trp Gly Glu Cys Gly Ala Glu Glu Pro Pro Ser Thr Pro Cys Leu Pro
                780                 785                 790

CGC TCC GGC CAC CTG GAC AAT AAC TGT GCC CGC CTC ACC TTG CAT TTC          2752
Arg Ser Gly His Leu Asp Asn Asn Cys Ala Arg Leu Thr Leu His Phe
            795                 800                 805
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AAC CGT GAC CAC GTG CCC CAG GGC ACC ACG GTG GGC GCC ATT TGC TCC      2800
Asn Arg Asp His Val Pro Gln Gly Thr Thr Val Gly Ala Ile Cys Ser
        810                 815                 820

GGG ATC CGC TCC CTG CCA GCC ACA AGG GCT GTG GCA CGG GAC CGC CTG      2848
Gly Ile Arg Ser Leu Pro Ala Thr Arg Ala Val Ala Arg Asp Arg Leu
    825                 830                 835

CTG GTG TTG CTT TGC GAC CGG GCG TCC TCG GGG GCC AGT GCT GTG GAG      2896
Leu Val Leu Leu Cys Asp Arg Ala Ser Ser Gly Ala Ser Ala Val Glu
840                 845                 850                 855

GTG GCC GTG TCC TTC AGC CCT GCC AGG GAC CTG CCT GAC AGC AGC CTG      2944
Val Ala Val Ser Phe Ser Pro Ala Arg Asp Leu Pro Asp Ser Ser Leu
                860                 865                 870

ATC CAG GGC GCG GCC CAC GCC ATC GTG GCC GCC ATC ACC CAG CGG GGG      2992
Ile Gln Gly Ala Ala His Ala Ile Val Ala Ala Ile Thr Gln Arg Gly
            875                 880                 885

AAC AGC TCA CTG CTC CTG GCT GTC ACC GAG GTC AAG GTG GAG ACG GTT      3040
Asn Ser Ser Leu Leu Leu Ala Val Thr Glu Val Lys Val Glu Thr Val
        890                 895                 900

GTT ACG GGC GGC TCT TCC ACA GGT CTG CTG GTG CCT GTG CTG TGT GGT      3088
Val Thr Gly Gly Ser Ser Thr Gly Leu Leu Val Pro Val Leu Cys Gly
    905                 910                 915

GCC TTC AGC GTG CTG TGG CTG GCG TGC GTG GTC CTG TGC GTG TGG TGG      3136
Ala Phe Ser Val Leu Trp Leu Ala Cys Val Val Leu Cys Val Trp Trp
920                 925                 930                 935

ACA CGC AAG CGC AGG AAA GAG CGG GAG AGG AGC CGG CTG CCG CGG GAG      3184
Thr Arg Lys Arg Arg Lys Glu Arg Glu Arg Ser Arg Leu Pro Arg Glu
                940                 945                 950

GAG AGC GCC AAC AAC CAG TGG GCC CCG CTC AAC CCC ATC CGC AAC CCC      3232
Glu Ser Ala Asn Asn Gln Trp Ala Pro Leu Asn Pro Ile Arg Asn Pro
            955                 960                 965

ATT GAG CGG CCG GGG GGG CAC AAG GAC GTG CTC TAC CAG TGC AAG AAC      3280
Ile Glu Arg Pro Gly Gly His Lys Asp Val Leu Tyr Gln Cys Lys Asn
        970                 975                 980

TTC ACT CCA CCG CCG CGC AGG CGC TGC CCG GGC CGG CCG GCC ACG CGG      3328
Phe Thr Pro Pro Pro Arg Arg Arg Cys Pro Gly Arg Pro Ala Thr Arg
    985                 990                 995

CCG TCA GGG AGG ATG AGG AGG ACG AGG ATC TTG GCC GCG GTG AGG AGG      3376
Pro Ser Gly Arg Met Arg Arg Thr Arg Ile Leu Ala Ala Val Arg Arg
1000                1005                1010                1015

ACT CCC TGG AGG CGG AGA AGT TCC TCT CAC ACA AAT TCA CCA AAG ATC      3424
Thr Pro Trp Arg Arg Arg Ser Ser Ser His Thr Asn Ser Pro Lys Ile
                1020                1025                1030

CTG GCC GCT CGC CGG GGA GGC CGG CCC ACT GGG CCT CAG GCC CCA AAG      3472
Leu Ala Ala Arg Arg Gly Gly Arg Pro Thr Gly Pro Gln Ala Pro Lys
            1035                1040                1045

TGG ACA ACC GCG CGG TCA GGA GCA TCA ATG AGG CCC GCT ACG TCG GCA      3520
Trp Thr Thr Ala Arg Ser Gly Ala Ser Met Arg Pro Ala Thr Ser Ala
        1050                1055                1060

AGG GAA GTA GGG CGG CTG CAG CTG GGC CGG GAC CCA GGG CCC TCG GTG      3568
Arg Glu Val Gly Arg Leu Gln L u Gly Arg Asp Pro Gly Pro Ser Val
    1065                1070                1075
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GGA GCC ATG CCG TCT GCC GGA CCC GGA GGC CGA GGC CAT GTG CAT AGT      3616
Gly Ala Met Pro Ser Ala Gly Pro Gly Gly Arg Gly His Val His Ser
1080                 1085                 1090                 1095

TTC TTT ATT TTG TGT AAA AAA ACC ACC AAA AAC AAA AAC CAA ATG TTT      3664
Phe Phe Ile Leu Cys Lys Lys Thr Thr Lys Asn Lys Asn Gln Met Phe
                1100                 1105                 1110

ATT TTC TAC GTT TCT TTA ACC TTG TAT AAA TTA TTC AGT AAC TGT CAG      3712
Ile Phe Tyr Val Ser Leu Thr Leu Tyr Lys Leu Phe Ser Asn Cys Gln
            1115                 1120                 1125

GCT GAA AAC AAT GGA GTA TTC TCG GAT AGT TGC TAT TTT TGT AAA GTA      3760
Ala Glu Asn Asn Gly Val Phe Ser Asp Ser Cys Tyr Phe Cys Lys Val
        1130                 1135                 1140

GCC GTG CGT GGC ACT CGC TGT ATG AAA GGA GAG AGC AAA GGG TGT CTG      3808
Ala Val Arg Gly Thr Arg Cys Met Lys Gly Glu Ser Lys Gly Cys Leu
    1145                 1150                 1155

CGT CGT CAC CAA ATC GTC GCG TTT GTT ACC AGA GGT TGT GCA CTG TTT      3856
Arg Arg His Gln Ile Val Ala Phe Val Thr Arg Gly Cys Ala Leu Phe
1160                 1165                 1170                 1175

ACA GAA TCT TCC TTT TAT TCC TCA CTC GGG TTT CTC TGT GCT CCA GGC      3904
Thr Glu Ser Ser Phe Tyr Ser Ser Leu Gly Phe Leu Cys Ala Pro Gly
                1180                 1185                 1190

CAA AGT GCC GGT GAG ACC CAT GGC TGT GTT GGT GTG GCC CAT GGC TGT      3952
Gln Ser Ala Gly Glu Thr His Gly Cys Val Gly Val Ala His Gly Cys
            1195                 1200                 1205

TGG TGG GAC CCG TGG CTG ATG GTG TGG CCT GTG GCT GTC GGT GGG ACT      4000
Trp Trp Asp Pro Trp Leu Met Val Trp Pro Val Ala Val Gly Gly Thr
        1210                 1215                 1220

CGT GGC TGT CAA TGG GAC CTG TGG CTG TCG GTG GGA CCT ACG GTG GTC      4048
Arg Gly Cys Gln Trp Asp Leu Trp Leu Ser Val Gly Pro Thr Val Val
    1225                 1230                 1235

GGT GGG ACC CTG GTT ATT GAT GTG GCC CTG GCT GCC GGC ACG GCC CGT      4096
Gly Gly Thr Leu Val Ile Asp Val Ala Leu Ala Ala Gly Thr Ala Arg
1240                 1245                 1250                 1255

GGC TGT TG ACGCACCT GTGGTTGTTA GTGGGGCCTG AGGTCATCGGC GTGGCCCAAG     4154
Gly Cys

GCCGGCAGGT CAACCTCGCG CTTGCTGGCC AGTCCACCCT GCCTGCCGTCT GTGCTTCCTC   4214

CTGCCCAGAA CGCCCGCTCC AGCGATCTCT CCACTGTGCT TTCAGAAGTGC CCTTCCTGCT   4274

GCGCAGTTCT CCCATCCTGG GACGGCGGCA GTATTGAAGC TCGTGACAAGT GCCTTCACAC   4334

AGACCCCTCG CAACTGTCCA CGCGTGCCGT GGCACCAGGC GCTGCCCACCT GCCGGCCCCG   4394

GCCGCCCCTC CTCGTGAAAG TGCATTTTTG TAAATGTGTA CATATTAAAGG AAGCACTCTG   4454

TATAAAAAAA AAAAACCGGA ATTCC                                          4483
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1384 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Ile Asn Pro Glu Asp Arg Trp Lys Ser Leu His Phe Ser Gly His
 1               5                  10                  15

Val Ala His Leu Glu Leu Gln Ile Arg Val Arg Cys Asp Glu Asn Tyr
            20                  25                  30

Tyr Ser Ala Thr Cys Asn Lys Phe Cys Arg Pro Arg Asn Asp Phe Phe
        35                  40                  45

Gly His Tyr Thr Cys Asp Gln Tyr Gly Asn Lys Ala Cys Met Asp Gly
    50                  55                  60

Trp Met Gly Lys Glu Cys Lys Glu Ala Val Cys Lys Gln Gly Cys Asn
65                  70                  75                  80

Leu Leu His Gly Gly Cys Thr Val Pro Gly Glu Cys Arg Cys Ser Tyr
                85                  90                  95

Gly Trp Gln Gly Arg Phe Cys Asp Glu Cys Val Pro Tyr Pro Gly Cys
            100                 105                 110

Val His Gly Ser Cys Val Glu Pro Trp Gln Cys Asn Cys Glu Thr Asn
        115                 120                 125

Trp Gly Gly Leu Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Ser His
    130                 135                 140

His Pro Cys Thr Asn Gly Gly Thr Cys Ile Asn Ala Glu Pro Asp Gln
145                 150                 155                 160

Tyr Arg Cys Thr Cys Pro Asp Gly Tyr Ser Gly Arg Asn Cys Glu Lys
                165                 170                 175

Ala Glu His Ala Cys Thr Ser Asn Pro Cys Ala Asn Gly Gly Ser Cys
            180                 185                 190

His Glu Val Pro Ser Gly Phe Glu Cys His Cys Pro Ser Gly Trp Ser
        195                 200                 205

Gly Pro Thr Cys Ala Leu Asp Ile Asp Glu Cys Ala Ser Asn Pro Cys
    210                 215                 220

Ala Ala Gly Gly Thr Cys Val Asp Gln Val Asp Gly Phe Glu Cys Ile
225                 230                 235                 240

Cys Pro Glu Gln Trp Val Gly Ala Thr Cys Gln Leu Asp Ala Asn Glu
                245                 250                 255

Cys Glu Gly Lys Pro Cys Leu Asn Ala Phe Ser Cys Lys Asn Leu Ile
            260                 265                 270

Gly Gly Tyr Tyr Cys Asp Cys Ile Pro Gly Trp Lys Gly Ile Asn Cys
        275                 280                 285

His Ile Asn Val Asn Asp Cys Arg Gly Gln Cys Gln His Gly Gly Thr
    290                 295                 300

Cys Lys Asp Leu Val Asn Gly Tyr Gln Cys Val Cys Pro Arg Gly Phe
305                 310                 315                 320

Gly Gly Arg His Cys Glu Leu Glu Arg Asp Lys Cys Ala Ser Ser Pro
                325                 330                 335
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Cys His Ser Gly Gly Leu Cys Glu Asp Leu Ala Asp Gly Phe His Cys
340 345 350

His Cys Pro Gln Gly Phe Ser Gly Pro Leu Cys Glu Val Asp Val Asp
355 360 365

Leu Cys Glu Pro Ser Pro Cys Arg Asn Gly Ala Arg Cys Tyr Asn Leu
370 375 380

Glu Gly Asp Tyr Tyr Cys Ala Cys Pro Asp Asp Phe Gly Gly Lys Asn
385 390 395 400

Cys Ser Val Pro Arg Glu Pro Cys Pro Gly Gly Ala Cys Arg Val Ile
405 410 415

Asp Gly Cys Gly Ser Asp Ala Gly Pro Gly Met Pro Gly Thr Ala Ala
420 425 430

Ser Gly Val Cys Gly Pro His Gly Arg Cys Val Ser Gln Pro Gly Gly
435 440 445

Asn Phe Ser Cys Ile Cys Asp Ser Gly Phe Thr Gly Thr Tyr Cys His
450 455 460

Glu Asn Ile Asp Asp Cys Leu Gly Gln Pro Cys Arg Asn Gly Gly Thr
465 470 475 480

Cys Ile Asp Glu Val Asp Ala Phe Arg Cys Phe Cys Pro Ser Gly Trp
485 490 495

Glu Gly Glu Leu Cys Asp Thr Asn Pro Asn Asp Cys Leu Pro Asp Pro
500 505 510

Cys His Ser Arg Gly Arg Cys Tyr Asp Leu Val Asn Asp Phe Tyr Cys
515 520 525

Ala Cys Asp Asp Gly Trp Lys Gly Lys Thr Cys His Ser Arg Glu Phe
530 535 540

Gln Cys Asp Ala Tyr Thr Cys Ser Asn Gly Gly Thr Cys Tyr Asp Ser
545 550 555 560

Gly Asp Thr Phe Arg Cys Ala Cys Pro Pro Gly Trp Lys Gly Ser Thr
565 570 575

Cys Ala Val Ala Lys Asn Ser Ser Cys Leu Pro Asn Pro Cys Val Asn
580 585 590

Gly Gly Thr Cys Val Gly Ser Gly Ala Ser Phe Ser Cys Ile Cys Arg
595 600 605

Asp Gly Trp Glu Gly Arg Thr Cys Thr His Asn Thr Asn Asp Cys Asn
610 615 620

Pro Leu Pro Cys Tyr Asn Gly Gly Ile Cys Val Asp Gly Val Asn Trp
625 630 635 640

Phe Arg Cys Glu Cys Ala Pro Gly Phe Ala Gly Pro Asp Cys Arg Ile
645 650 655

Asn Ile Asp Glu Cys Gln Ser Ser Pro Cys Ala Tyr Gly Ala Thr Cys
660 665 670

Val Asp Glu Ile Asn Gly Tyr Arg Cys Ser Cys Pro Pro Gly Arg Ala
675 680 685

Gly Pro Arg Cys Gln Glu Val Ile Gly Phe Gly Arg Ser Cys Trp Ser

690 695 700

Arg Gly Thr Pro Phe Pro His Gly Ser Ser Trp Val Glu Asp Cys Asn
705 710 715 720

Ser Cys Arg Cys Leu Asp Gly Arg Arg Asp Cys Ser Lys Val Trp Cys
725 730 735

Gly Trp Lys Pro Cys Leu Leu Ala Gly Gln Pro Glu Ala Leu Ser Ala
740 745 750

Gln Cys Pro Leu Gly Gln Arg Cys Leu Glu Lys Ala Pro Gly Gln Cys
755 760 765

Leu Arg Pro Pro Cys Glu Ala Trp Gly Glu Cys Gly Ala Glu Glu Pro
770 775 780

Pro Ser Thr Pro Cys Leu Pro Arg Ser Gly His Leu Asp Asn Asn Cys
785 790 795 800

Ala Arg Leu Thr Leu His Phe Asn Arg Asp His Val Pro Gln Gly Thr
805 810 815

Thr Val Gly Ala Ile Cys Ser Gly Ile Arg Ser Leu Pro Ala Thr Arg
820 825 830

Ala Val Ala Arg Asp Arg Leu Leu Val Leu Leu Cys Asp Arg Ala Ser
835 840 845

Ser Gly Ala Ser Ala Val Glu Val Ala Val Ser Phe Ser Pro Ala Arg
850 855 860

Asp Leu Pro Asp Ser Ser Leu Ile Gln Gly Ala Ala His Ala Ile Val
865 870 875 880

Ala Ala Ile Thr Gln Arg Gly Asn Ser Ser Leu Leu Leu Ala Val Thr
885 890 895

Glu Val Lys Val Glu Thr Val Val Thr Gly Gly Ser Ser Thr Gly Leu
900 905 910

Leu Val Pro Val Leu Cys Gly Ala Phe Ser Val Leu Trp Leu Ala Cys
915 920 925

Val Val Leu Cys Val Trp Trp Thr Arg Lys Arg Arg Lys Glu Arg Glu
930 935 940

Arg Ser Arg Leu Pro Arg Glu Glu Ser Ala Asn Asn Gln Trp Ala Pro
945 950 955 960

Leu Asn Pro Ile Arg Asn Pro Ile Glu Arg Pro Gly Gly His Lys Asp
965 970 975

Val Leu Tyr Gln Cys Lys Asn Phe Thr Pro Pro Pro Arg Arg Arg Cys
980 985 990

Pro Gly Arg Pro Ala Thr Arg Pro Ser Gly Arg Met Arg Arg Thr Arg
995 1000 1005

Ile Leu Ala Ala Val Arg Arg Thr Pro Trp Arg Arg Arg Ser Ser Ser
1010 1015 1020

His Thr Asn Ser Pro Lys Ile Leu Ala Ala Arg Arg Gly Gly Arg Pro
1025 1030 1035 1040

Thr Gly Pro Gln Ala Pr Lys Trp Thr Thr Ala Arg Ser Gly Ala Ser
1045 1050 1055

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Met Arg Pro Ala Thr Ser Ala Arg Glu Val Gly Arg Leu Gln Leu Gly
            1060                1065                1070

Arg Asp Pro Gly Pro Ser Val Gly Ala Met Pro Ser Ala Gly Pro Gly
        1075                1080                1085

Gly Arg Gly His Val His Ser Phe Phe Ile Leu Cys Lys Lys Thr Thr
    1090                1095                1100

Lys Asn Lys Asn Gln Met Phe Ile Phe Tyr Val Ser Leu Thr Leu Tyr
1105                1110                1115                1120

Lys Leu Phe Ser Asn Cys Gln Ala Glu Asn Asn Gly Val Phe Ser Asp
                1125                1130                1135

Ser Cys Tyr Phe Cys Lys Val Ala Val Arg Gly Thr Arg Cys Met Lys
            1140                1145                1150

Gly Glu Ser Lys Gly Cys Leu Arg Arg His Gln Ile Val Ala Phe Val
        1155                1160                1165

Thr Arg Gly Cys Ala Leu Phe Thr Glu Ser Ser Phe Tyr Ser Ser Leu
    1170                1175                1180

Gly Phe Leu Cys Ala Pro Gly Gln Ser Ala Gly Glu Thr His Gly Cys
1185                1190                1195                1200

Val Gly Val Ala His Gly Cys Trp Trp Asp Pro Trp Leu Met Val Trp
                1205                1210                1215

Pro Val Ala Val Gly Gly Thr Arg Gly Cys Gln Trp Asp Leu Trp Leu
            1220                1225                1230

Ser Val Gly Pro Thr Val Val Gly Gly Thr Leu Val Ile Asp Val Ala
        1235                1240                1245

Leu Ala Ala Gly Thr Ala Arg Gly Cys
    1250                1255
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3582 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..3582

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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CAG GTG GCG TCA GCA TCG GGA CAG TTC GAG CTG GAG ATC TTA TCC GTG      48
Gln Val Ala Ser Ala Ser Gly Gln Phe Glu Leu Glu Ile Leu Ser Val
  1               5                  10                  15

CAG AAT GTG AAC GGC GTG CTG CAG AAC GGG AAC TGC TGC GAC GGC ACT      96
Gln Asn Val Asn Gly Val Leu Gln Asn Gly Asn Cys Cys Asp Gly Thr
            20                  25                  30
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CGA AAC CCC GGA GAT AAA AAG TGC ACC AGA GAT GAG TGT GAC ACC TAC          144
Arg Asn Pro Gly Asp Lys Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr
          35                  40                  45

TTT AAA GTT TGC CTG AAG GAG TAC CAG TCG CGG GTC ACT GCT GGC GGC          192
Phe Lys Val Cys Leu Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly
      50                  55                  60

CCT TGC AGC TTC GGA TCC AAA TCC ACC CCT GTC ATC GGC GGG AAT ACC          240
Pro Cys Ser Phe Gly Ser Lys Ser Thr Pro Val Ile Gly Gly Asn Thr
 65                  70                  75                  80

TTC AAT TTA AAG TAC AGC CGG AAT AAT GAA AAG AAC CGG ATT GTT ATC          288
Phe Asn Leu Lys Tyr Ser Arg Asn Asn Glu Lys Asn Arg Ile Val Ile
                 85                  90                  95

CCT TTC ACG TTC GCC TGG CCG AGA TCC TAC ACG TTG CTT GTT GAG GCA          336
Pro Phe Thr Phe Ala Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala
            100                 105                 110

TGG GAT TAC AAT GAT AAC TCT ACT AAT CCC GAT CGC ATA ATT GAG AAG          384
Trp Asp Tyr Asn Asp Asn Ser Thr Asn Pro Asp Arg Ile Ile Glu Lys
        115                 120                 125

GCA TCC CAC TCT GGC ATG ATC AAT CCA AGC CGT CAG TGG CAG ACG TTG          432
Ala Ser His Ser Gly Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu
    130                 135                 140

AAA CAT AAC ACA GGA GCT GCC CAC TTT GAG TAT CAA ATC CGT GTG ACT          480
Lys His Asn Thr Gly Ala Ala His Phe Glu Tyr Gln Ile Arg Val Thr
145                 150                 155                 160

TGC GCA GAA CAT TAC TAT GGC TTT GGA TGC AAC AAG TTT TGT CGA CCG          528
Cys Ala Glu His Tyr Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro
                165                 170                 175

AGA GAT GAC TTC TTC ACT CAC CAT ACC TGT GAC CAG AAT GGC AAC AAA          576
Arg Asp Asp Phe Phe Thr His His Thr Cys Asp Gln Asn Gly Asn Lys
            180                 185                 190

ACC TGC TTG GAA GGC TGG ACG GGA CCA GAA TGC AAC AAA GCT ATT TGT          624
Thr Cys Leu Glu Gly Trp Thr Gly Pro Glu Cys Asn Lys Ala Ile Cys
        195                 200                 205

CGT CAG GGA TGT AGC CCC AAG CAT GGT TCT TGC ACA GTT CCA GGA GAG          672
Arg Gln Gly Cys Ser Pro Lys His Gly Ser Cys Thr Val Pro Gly Glu
    210                 215                 220

TGC AGG TGT CAG TAT GGA TGG CAA GGC CAG TAC TGT GAT AAG TGC ATT          720
Cys Arg Cys Gln Tyr Gly Trp Gln Gly Gln Tyr Cys Asp Lys Cys Ile
225                 230                 235                 240

CCA CAC CCG GGA TGT GTC CAT GGC ACT TGC ATT GAA CCA TGG CAG TGC          768
Pro His Pro Gly Cys Val His Gly Thr Cys Ile Glu Pro Trp Gln Cys
                245                 250                 255

CTC TGT GAA ACC AAC TGG GGT GGT CAG CTC TGT GAC AAA GAC CTG AAC          816
Leu Cys Glu Thr Asn Trp Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn
            260                 265                 270

TAC TGT GGA ACC CAC CCA CCC TGT TTG AAT GGT GGT ACC TGC AGC AAC          864
Tyr Cys Gly Thr His Pro Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn
        275                 280                 285

ACT GGC CCC GAT AAA TAC CAG TGT TCC TGC CCT GAG GGT TAC TCA GGA          912
Thr Gly Pro Asp Lys Tyr Gln Cys Ser Cys Pro Glu Gly Tyr S r Gly
    290                 295                 300
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CAG AAC TGT GAA ATA GCG GAG CAT GCG TGC CTC TCT GAT CCG TGC CAC          960
Gln Asn Cys Glu Il  Ala Glu His Ala Cys Leu Ser Asp Pro Cys His
305                 310                 315                 320

AAC GGA GGA AGC TGC CTA GAA ACG TCT ACA GGA TTT GAA TGT GTG TGT         1008
Asn Gly Gly Ser Cys Leu Glu Thr Ser Thr Gly Phe Glu Cys Val Cys
                    325                 330                 335

GCA CCT GGC TGG GCT GGA CCA ACT TGC ACT GAT AAT ATT GAT GAT TGT         1056
Ala Pro Gly Trp Ala Gly Pro Thr Cys Thr Asp Asn Ile Asp Asp Cys
                340                 345                 350

TCT CCA AAT CCC TGT GGT CAT GGA GGA ACT TGC CAA GAT CTA GTT GAT         1104
Ser Pro Asn Pro Cys Gly His Gly Gly Thr Cys Gln Asp Leu Val Asp
            355                 360                 365

GGA TTT AAG TGT ATT TGC CCA CCT CAG TGG ACT GGC AAA ACA TGC CAG         1152
Gly Phe Lys Cys Ile Cys Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln
    370                 375                 380

CTA GAT GCG AAT GAA TGT GAG GGC AAA CCC TGT GTC AAT GCC AAC TCC         1200
Leu Asp Ala Asn Glu Cys Glu Gly Lys Pro Cys Val Asn Ala Asn Ser
385                 390                 395                 400

TGC AGG AAC TTG ATT GGC AGC TAC TAT TGT GAC TGC ATT ACT GGC TGG         1248
Cys Arg Asn Leu Ile Gly Ser Tyr Tyr Cys Asp Cys Ile Thr Gly Trp
                405                 410                 415

TCT GGC CAC AAC TGT GAT ATA AAT ATT AAT GAT TGT CGT GGA CAA TGT         1296
Ser Gly His Asn Cys Asp Ile Asn Ile Asn Asp Cys Arg Gly Gln Cys
            420                 425                 430

CAG AAT GGA GGA TCC TGT CGG GAC TTG GTT AAT GGT TAT CGG TGC ATC         1344
Gln Asn Gly Gly Ser Cys Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile
        435                 440                 445

TGT TCA CCT GGC TAT GCA GGA GAT CAC TGT GAG AAA GAC ATC AAT GAA         1392
Cys Ser Pro Gly Tyr Ala Gly Asp His Cys Glu Lys Asp Ile Asn Glu
    450                 455                 460

TGT GCA AGT AAC CCT TGC ATG AAT GGG GGT CAC TGC CAG GAT GAA ATC         1440
Cys Ala Ser Asn Pro Cys Met Asn Gly Gly His Cys Gln Asp Glu Ile
465                 470                 475                 480

AAT GGA TTC CAA TGT CTG TGT CCT GCT GGT TTC TCA GGA AAC CTC TGT         1488
Asn Gly Phe Gln Cys Leu Cys Pro Ala Gly Phe Ser Gly Asn Leu Cys
                485                 490                 495

CAG CTG GAT ATA GAC TAC TGT GAG CCA AAC CCT TGC CAG AAC GGT GCC         1536
Gln Leu Asp Ile Asp Tyr Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala
            500                 505                 510

CAG TGC TTC AAT CTT GCT ATG GAC TAT TTC TGT AAC TGC CCT GAA GAT         1584
Gln Cys Phe Asn Leu Ala Met Asp Tyr Phe Cys Asn Cys Pro Glu Asp
        515                 520                 525

TAC GAA GGC AAG AAC TGC TCC CAC CTG AAA GAT CAC TGC CGC ACA ACT         1632
Tyr Glu Gly Lys Asn Cys Ser His Leu Lys Asp His Cys Arg Thr Thr
    530                 535                 540

CCT TGT GAA GTA ATC GAC AGC TGT ACA GTG GCA GTG GCT TCT AAC AGC         1680
Pro Cys Glu Val Ile Asp Ser Cys Thr Val Ala Val Ala Ser Asn Ser
545                 550                 555                 560

ACA CCA GAA GGA GTT CGT TAC ATT TCT TCA AAT GTC TGT GGT CCT CAT         1728
Thr Pro Glu Gly Val Arg Tyr Ile S r Ser Asn Val Cys Gly Pro His
                565                 570                 575
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GGA AAA TGC AAG AGC CAA GCA GGT GGA AAA TTC ACC TGT GAA TGC AAC      1776
Gly Lys Cys Lys S r Gln Ala Gly Gly Lys Phe Thr Cys Glu Cys Asn
            580                 585                 590

AAA GGA TTC ACT GGC ACC TAC TGT CAT GAG AAT ATC AAT GAC TGT GAG      1824
Lys Gly Phe Thr Gly Thr Tyr Cys His Glu Asn Ile Asn Asp Cys Glu
        595                 600                 605

AGC AAC CCC TGT AAA AAT GGT GGC ACT TGT ATT GAC GGT GTA AAC TCC      1872
Ser Asn Pro Cys Lys Asn Gly Gly Thr Cys Ile Asp Gly Val Asn Ser
    610                 615                 620

TAC AAA TGT ATT TGT AGT GAT GGA TGG GAA GGA ACA TAT TGT GAA ACA      1920
Tyr Lys Cys Ile Cys Ser Asp Gly Trp Glu Gly Thr Tyr Cys Glu Thr
625                 630                 635                 640

AAT ATT AAT GAC TGC AGT AAA AAC CCC TGC CAC AAT GGA GGA ACT TGC      1968
Asn Ile Asn Asp Cys Ser Lys Asn Pro Cys His Asn Gly Gly Thr Cys
                645                 650                 655

CGA GAC TTG GTC AAT GAC TTC TTC TGT GAA TGT AAA AAT GGG TGG AAA      2016
Arg Asp Leu Val Asn Asp Phe Phe Cys Glu Cys Lys Asn Gly Trp Lys
            660                 665                 670

GGA AAA ACT TGC CAC TCT CGT GAC AGC CAG TGT GAT GAG GCA ACA TGC      2064
Gly Lys Thr Cys His Ser Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys
        675                 680                 685

AAT AAT GGA GGA ACA TGT TAT GAT GAG GGG GAC ACT TTC AAG TGC ATG      2112
Asn Asn Gly Gly Thr Cys Tyr Asp Glu Gly Asp Thr Phe Lys Cys Met
    690                 695                 700

TGT CCT GCA GGA TGG GAA GGA GCC ACT TGT AAT ATA GCA AGG AAC AGC      2160
Cys Pro Ala Gly Trp Glu Gly Ala Thr Cys Asn Ile Ala Arg Asn Ser
705                 710                 715                 720

AGC TGC CTG CCA AAC CCC TGT CAC AAT GGT GGT ACC TGT GTA GTT AGT      2208
Ser Cys Leu Pro Asn Pro Cys His Asn Gly Gly Thr Cys Val Val Ser
                725                 730                 735

GGG GAT TCT TTC ACT TGT GTC TGC AAG GAG GGC TGG GAA GGA CCG ACA      2256
Gly Asp Ser Phe Thr Cys Val Cys Lys Glu Gly Trp Glu Gly Pro Thr
            740                 745                 750

TGT ACT CAG AAC ACA AAT GAC TGC AGT CCT CAT CCT TGT TAC AAC AGT      2304
Cys Thr Gln Asn Thr Asn Asp Cys Ser Pro His Pro Cys Tyr Asn Ser
        755                 760                 765

GGT ACT TGT GTG GAT GGA GAC AAC TGG TAC CGC TGT GAG TGC GCT CCC      2352
Gly Thr Cys Val Asp Gly Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro
    770                 775                 780

GGC TTC GCA GGT CCC GAC TGT AGG ATC AAC ATC AAT GAA TGT CAG TCT      2400
Gly Phe Ala Gly Pro Asp Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser
785                 790                 795                 800

TCA CCC TGT GCC TTT GGG GCT ACT TGT GTG GAT GAA ATT AAT GGG TAC      2448
Ser Pro Cys Ala Phe Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr
                805                 810                 815

CGT TGC ATT TGT CCA CCG GGT CGC AGT GGT CCA GGA TGC CAG GAA GTT      2496
Arg Cys Ile Cys Pro Pro Gly Arg Ser Gly Pro Gly Cys Gln Glu Val
            820                 825                 830

ACA GGG AGG CCT TGC TTT ACC AGT ATT CGA GTA ATG CCA GAC GGT GCT      2544
Thr Gly Arg Pro Cys Phe Thr Ser Ile Arg Val Met Pro Asp Gly Ala
        835                 840                 845
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AAG TGG GAT GAT GAC TGT AAT ACT TGT CAG TGT TTG AAT GGA AAA GTC          2592
Lys Trp Asp Asp Asp Cys Asn Thr Cys Gln Cys Leu Asn Gly Lys Val
    850                 855                 860

ACC TGT TCT AAG GTT TGG TGT GGT CCT CGA CCT TGT ATA ATA CAT GCC          2640
Thr Cys Ser Lys Val Trp Cys Gly Pro Arg Pro Cys Ile Ile His Ala
865                 870                 875                 880

AAA GGT CAT AAT GAA TGC CCA GCT GGA CAC GCT TGT GTT CCT GTT AAA          2688
Lys Gly His Asn Glu Cys Pro Ala Gly His Ala Cys Val Pro Val Lys
                885                 890                 895

GAA GAC CAT TGT TTC ACT CAT CCT TGT GCT GCA GTG GGT GAA TGC TGG          2736
Glu Asp His Cys Phe Thr His Pro Cys Ala Ala Val Gly Glu Cys Trp
            900                 905                 910

CCT TCT AAT CAG CAG CCT GTG AAG ACC AAA TGC AAT TCT GAT TCT TAT          2784
Pro Ser Asn Gln Gln Pro Val Lys Thr Lys Cys Asn Ser Asp Ser Tyr
        915                 920                 925

TAC CAA GAT AAT TGT GCC AAC ATC ACC TTC ACC TTT AAT AAG GAA ATG          2832
Tyr Gln Asp Asn Cys Ala Asn Ile Thr Phe Thr Phe Asn Lys Glu Met
    930                 935                 940

ATG GCA CCA GGC CTT ACC ACG GAG CAC ATT TGC AGT GAA TTG AGG AAT          2880
Met Ala Pro Gly Leu Thr Thr Glu His Ile Cys Ser Glu Leu Arg Asn
945                 950                 955                 960

CTG AAT ATC CTG AAG AAT GTT TCT GCT GAA TAT TCC ATC TAT ATT ACC          2928
Leu Asn Ile Leu Lys Asn Val Ser Ala Glu Tyr Ser Ile Tyr Ile Thr
                965                 970                 975

TGT GAG CCT TCA CAC TTG GCA AAT AAT GAA ATA CAT GTT GCT ATT TCT          2976
Cys Glu Pro Ser His Leu Ala Asn Asn Glu Ile His Val Ala Ile Ser
            980                 985                 990

GCT GAA GAT ATA GGA GAA GAT GAA AAC CCA ATC AAG GAA ATC ACA GAT          3024
Ala Glu Asp Ile Gly Glu Asp Glu Asn Pro Ile Lys Glu Ile Thr Asp
        995                 1000                1005

AAG ATT ATT GAC CTT GTC AGT AAG CGT GAT GGA AAC AAC ACA CTA ATT          3072
Lys Ile Ile Asp Leu Val Ser Lys Arg Asp Gly Asn Asn Thr Leu Ile
    1010                1015                1020

GCT GCA GTC GCA GAA GTC AGA GTA CAA AGG CGA CCA GTT AAG AAC AAA          3120
Ala Ala Val Ala Glu Val Arg Val Gln Arg Arg Pro Val Lys Asn Lys
1025                1030                1035                1040

ACA GAT TTC TTG GTG CCA TTA CTG AGC TCA GTC TTA ACA GTA GCC TGG          3168
Thr Asp Phe Leu Val Pro Leu Leu Ser Ser Val Leu Thr Val Ala Trp
                1045                1050                1055

ATC TGC TGT CTG GTA ACT GTT TTC TAT TGG TGC ATT CAA AAG CGC AGA          3216
Ile Cys Cys Leu Val Thr Val Phe Tyr Trp Cys Ile Gln Lys Arg Arg
            1060                1065                1070

AAG CAG AGC AGC CAT ACT CAC ACA GCA TCT GAT GAC AAC ACC ACC AAC          3264
Lys Gln Ser Ser His Thr His Thr Ala Ser Asp Asp Asn Thr Thr Asn
        1075                1080                1085

AAC GTA AGG GAG CAG CTG AAT CAG ATT AAA AAC CCC ATA GAG AAA CAC          3312
Asn Val Arg Glu Gln Leu Asn Gln Ile Lys Asn Pro Ile Glu Lys His
    1090                1095                1100

GGA GCA AAT ACT GTT CCA ATT AAA GAC TAT GAA AAC AAA AAC TCT AAA          3360
Gly Ala Asn Thr Val Pro Ile Lys Asp Tyr Glu Asn Lys Asn Ser Lys
1105                1110                1115                1120
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ATC GCC AAA ATA AGG ACG CAC AAT TCA GAA GTG GAG GAA GAT GAC ATG      3408
Ile Ala Lys Ile Arg Thr His Asn Ser Glu Val Glu Glu Asp Asp Met
                1125                1130                1135

GAC AAA CAC CAG CAA AAG GCC CGG TTT GCC AAG CAG CCA GCG TAC ACT      3456
Asp Lys His Gln Gln Lys Ala Arg Phe Ala Lys Gln Pro Ala Tyr Thr
            1140                1145                1150

TTG GTA GAC AGA GAT GAA AAG CCA CCC AAC AGC ACA CCC ACA AAA CAC      3504
Leu Val Asp Arg Asp Glu Lys Pro Pro Asn Ser Thr Pro Thr Lys His
        1155                1160                1165

CCA AAC TGG ACA AAT AAA CAG GAC AAC AGA GAC TTG GAA AGT GCA CAA      3552
Pro Asn Trp Thr Asn Lys Gln Asp Asn Arg Asp Leu Glu Ser Ala Gln
    1170                1175                1180

AGT TTA AAT AGA ATG GAG TAC ATT GTA
Ser Leu Asn Arg Met Glu Tyr Ile Val
1185                1190
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1194 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Gln Val Ala Ser Ala Ser Gly Gln Phe Glu Leu Glu Ile Leu Ser Val
  1               5                  10                  15

Gln Asn Val Asn Gly Val Leu Gln Asn Gly Asn Cys Cys Asp Gly Thr
            20                  25                  30

Arg Asn Pro Gly Asp Lys Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr
        35                  40                  45

Phe Lys Val Cys Leu Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly
    50                  55                  60

Pro Cys Ser Phe Gly Ser Lys Ser Thr Pro Val Ile Gly Gly Asn Thr
65                  70                  75                  80

Phe Asn Leu Lys Tyr Ser Arg Asn Asn Glu Lys Asn Arg Ile Val Ile
                85                  90                  95

Pro Phe Thr Phe Ala Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala
            100                 105                 110

Trp Asp Tyr Asn Asp Asn Ser Thr Asn Pro Asp Arg Ile Ile Glu Lys
        115                 120                 125

Ala Ser His Ser Gly Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu
    130                 135                 140

Lys His Asn Thr Gly Ala Ala His Phe Glu Tyr Gln Ile Arg Val Thr
145                 150                 155                 160

Cys Ala Glu His Tyr Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro
                165                 170                 175
```

Arg Asp Asp Phe Phe Thr His His Thr Cys Asp Gln Asn Gly Asn Lys
180 185 190

Thr Cys Leu Glu Gly Trp Thr Gly Pro Glu Cys Asn Lys Ala Ile Cys
195 200 205

Arg Gln Gly Cys Ser Pro Lys His Gly Ser Cys Thr Val Pro Gly Glu
210 215 220

Cys Arg Cys Gln Tyr Gly Trp Gln Gly Gln Tyr Cys Asp Lys Cys Ile
225 230 235 240

Pro His Pro Gly Cys Val His Gly Thr Cys Ile Glu Pro Trp Gln Cys
245 250 255

Leu Cys Glu Thr Asn Trp Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn
260 265 270

Tyr Cys Gly Thr His Pro Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn
275 280 285

Thr Gly Pro Asp Lys Tyr Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly
290 295 300

Gln Asn Cys Glu Ile Ala Glu His Ala Cys Leu Ser Asp Pro Cys His
305 310 315 320

Asn Gly Gly Ser Cys Leu Glu Thr Ser Thr Gly Phe Glu Cys Val Cys
325 330 335

Ala Pro Gly Trp Ala Gly Pro Thr Cys Thr Asp Asn Ile Asp Asp Cys
340 345 350

Ser Pro Asn Pro Cys Gly His Gly Gly Thr Cys Gln Asp Leu Val Asp
355 360 365

Gly Phe Lys Cys Ile Cys Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln
370 375 380

Leu Asp Ala Asn Glu Cys Glu Gly Lys Pro Cys Val Asn Ala Asn Ser
385 390 395 400

Cys Arg Asn Leu Ile Gly Ser Tyr Tyr Cys Asp Cys Ile Thr Gly Trp
405 410 415

Ser Gly His Asn Cys Asp Ile Asn Ile Asn Asp Cys Arg Gly Gln Cys
420 425 430

Gln Asn Gly Gly Ser Cys Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile
435 440 445

Cys Ser Pro Gly Tyr Ala Gly Asp His Cys Glu Lys Asp Ile Asn Glu
450 455 460

Cys Ala Ser Asn Pro Cys Met Asn Gly Gly His Cys Gln Asp Glu Ile
465 470 475 480

Asn Gly Phe Gln Cys Leu Cys Pro Ala Gly Phe Ser Gly Asn Leu Cys
485 490 495

Gln Leu Asp Ile Asp Tyr Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala
500 505 510

Gln Cys Phe Asn Leu Ala Met Asp Tyr Phe Cys Asn Cys Pr Glu Asp
515 520 525

Tyr Glu Gly Lys Asn Cys Ser His Leu Lys Asp His Cys Arg Thr Thr

530 535 540

Pro Cys Glu Val Ile Asp Ser Cys Thr Val Ala Val Ala Ser Asn Ser
545 550 555 560

Thr Pro Glu Gly Val Arg Tyr Ile Ser Ser Asn Val Cys Gly Pro His
565 570 575

Gly Lys Cys Lys Ser Gln Ala Gly Gly Lys Phe Thr Cys Glu Cys Asn
580 585 590

Lys Gly Phe Thr Gly Thr Tyr Cys His Glu Asn Ile Asn Asp Cys Glu
595 600 605

Ser Asn Pro Cys Lys Asn Gly Gly Thr Cys Ile Asp Gly Val Asn Ser
610 615 620

Tyr Lys Cys Ile Cys Ser Asp Gly Trp Glu Gly Thr Tyr Cys Glu Thr
625 630 635 640

Asn Ile Asn Asp Cys Ser Lys Asn Pro Cys His Asn Gly Gly Thr Cys
645 650 655

Arg Asp Leu Val Asn Asp Phe Phe Cys Glu Cys Lys Asn Gly Trp Lys
660 665 670

Gly Lys Thr Cys His Ser Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys
675 680 685

Asn Asn Gly Gly Thr Cys Tyr Asp Glu Gly Asp Thr Phe Lys Cys Met
690 695 700

Cys Pro Ala Gly Trp Glu Gly Ala Thr Cys Asn Ile Ala Arg Asn Ser
705 710 715 720

Ser Cys Leu Pro Asn Pro Cys His Asn Gly Gly Thr Cys Val Val Ser
725 730 735

Gly Asp Ser Phe Thr Cys Val Cys Lys Glu Gly Trp Glu Gly Pro Thr
740 745 750

Cys Thr Gln Asn Thr Asn Asp Cys Ser Pro His Pro Cys Tyr Asn Ser
755 760 765

Gly Thr Cys Val Asp Gly Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro
770 775 780

Gly Phe Ala Gly Pro Asp Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser
785 790 795 800

Ser Pro Cys Ala Phe Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr
805 810 815

Arg Cys Ile Cys Pro Pro Gly Arg Ser Gly Pro Gly Cys Gln Glu Val
820 825 830

Thr Gly Arg Pro Cys Phe Thr Ser Ile Arg Val Met Pro Asp Gly Ala
835 840 845

Lys Trp Asp Asp Asp Cys Asn Thr Cys Gln Cys Leu Asn Gly Lys Val
850 855 860

Thr Cys Ser Lys Val Trp Cys Gly Pro Arg Pro Cys Ile Ile His Ala
865 870 875 880

Lys Gly His Asn Glu Cys Pro Ala Gly His Ala Cys Val Pro Val Lys
885 890 895

Glu Asp His Cys Phe Thr His Pro Cys Ala Ala Val Gly Glu Cys Trp
900 905 910

Pro Ser Asn Gln Gln Pro Val Lys Thr Lys Cys Asn Ser Asp Ser Tyr
915 920 925

Tyr Gln Asp Asn Cys Ala Asn Ile Thr Phe Thr Phe Asn Lys Glu Met
930 935 940

Met Ala Pro Gly Leu Thr Thr Glu His Ile Cys Ser Glu Leu Arg Asn
945 950 955 960

Leu Asn Ile Leu Lys Asn Val Ser Ala Glu Tyr Ser Ile Tyr Ile Thr
965 970 975

Cys Glu Pro Ser His Leu Ala Asn Asn Glu Ile His Val Ala Ile Ser
980 985 990

Ala Glu Asp Ile Gly Glu Asp Glu Asn Pro Ile Lys Glu Ile Thr Asp
995 1000 1005

Lys Ile Ile Asp Leu Val Ser Lys Arg Asp Gly Asn Asn Thr Leu Ile
1010 1015 1020

Ala Ala Val Ala Glu Val Arg Val Gln Arg Arg Pro Val Lys Asn Lys
1025 1030 1035 1040

Thr Asp Phe Leu Val Pro Leu Leu Ser Ser Val Leu Thr Val Ala Trp
1045 1050 1055

Ile Cys Cys Leu Val Thr Val Phe Tyr Trp Cys Ile Gln Lys Arg Arg
1060 1065 1070

Lys Gln Ser Ser His Thr His Thr Ala Ser Asp Asp Asn Thr Thr Asn
1075 1080 1085

Asn Val Arg Glu Gln Leu Asn Gln Ile Lys Asn Pro Ile Glu Lys His
1090 1095 1100

Gly Ala Asn Thr Val Pro Ile Lys Asp Tyr Glu Asn Lys Asn Ser Lys
1105 1110 1115 1120

Ile Ala Lys Ile Arg Thr His Asn Ser Glu Val Glu Glu Asp Asp Met
1125 1130 1135

Asp Lys His Gln Gln Lys Ala Arg Phe Ala Lys Gln Pro Ala Tyr Thr
1140 1145 1150

Leu Val Asp Arg Asp Glu Lys Pro Pro Asn Ser Thr Pro Thr Lys His
1155 1160 1165

Pro Asn Trp Thr Asn Lys Gln Asp Asn Arg Asp Leu Glu Ser Ala Gln
1170 1175 1180

Ser Leu Asn Arg Met Glu Tyr Ile Val
1185 1190

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 236 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met His Trp Ile Lys Cys Leu Leu Thr Ala Phe Ile Cys Phe Thr Val
 1               5                  10                  15

Ile Val Gln Val His Ser Ser Gly Ser Phe Glu Leu Arg Leu Lys Tyr
            20                  25                  30

Phe Ser Asn Asp His Gly Arg Asp Asn Glu Gly Arg Cys Cys Ser Gly
        35                  40                  45

Glu Ser Asp Gly Ala Thr Gly Lys Cys Leu Gly Ser Cys Lys Thr Arg
    50                  55                  60

Phe Arg Val Cys Leu Lys His Tyr Gln Ala Thr Ile Asp Thr Thr Ser
65                  70                  75                  80

Gln Cys Thr Tyr Gly Asp Val Ile Thr Pro Ile Leu Gly Glu Asn Ser
                85                  90                  95

Val Asn Leu Thr Asp Ala Gln Arg Phe Gln Asn Lys Gly Phe Thr Asn
            100                 105                 110

Pro Ile Gln Phe Pro Phe Ser Phe Ser Trp Pro Gly Thr Phe Ser Leu
        115                 120                 125

Ile Val Glu Ala Trp His Asp Thr Asn Asn Ser Gly Asn Ala Arg Thr
    130                 135                 140

Asn Lys Leu Leu Ile Gln Arg Leu Leu Val Gln Gln Val Leu Glu Val
145                 150                 155                 160

Ser Ser Glu Trp Lys Thr Asn Lys Ser Glu Ser Gln Tyr Thr Ser Leu
                165                 170                 175

Glu Tyr Asp Phe Arg Val Thr Cys Asp Leu Asn Tyr Tyr Gly Ser Gly
            180                 185                 190

Cys Ala Lys Phe Cys Arg Pro Arg Asp Asp Ser Phe Gly His Ser Thr
        195                 200                 205

Cys Ser Glu Thr Gly Glu Ile Ile Cys Leu Thr Gly Trp Gln Gly Asp
    210                 215                 220

Tyr Cys His Ile Pro Lys Cys Ala Lys Gly Cys Glu
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1405 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Phe Arg Lys His Phe Arg Arg Lys Pro Ala Thr Ser Ser Ser Leu
 1               5                  10                  15

Glu Ser Thr Ile Glu Ser Ala Asp Ser Leu Gly Met Ser Lys Lys Thr
            20                  25                  30

Ala Thr Lys Arg Gln Arg Pro Arg His Arg Val Pro Lys Ile Ala Thr
        35                  40                  45

Leu Pro Ser Thr Ile Arg Asp Cys Arg Ser Leu Lys Ser Ala Cys Asn
    50                  55                  60
```

Leu Ile Ala Leu Ile Leu Ile Leu Leu Val His Lys Ile Ser Ala Ala
65 70 75 80

Gly Asn Phe Glu Leu Glu Ile Leu Glu Ile Ser Asn Thr Asn Ser His
85 90 95

Leu Leu Asn Gly Tyr Cys Cys Gly Met Pro Ala Glu Leu Arg Ala Thr
100 105 110

Lys Thr Ile Gly Cys Ser Pro Cys Thr Thr Ala Phe Arg Leu Cys Leu
115 120 125

Lys Glu Tyr Gln Thr Thr Glu Gln Gly Ala Ser Ile Ser Thr Gly Cys
130 135 140

Ser Phe Gly Asn Ala Thr Thr Lys Ile Leu Gly Gly Ser Ser Phe Val
145 150 155 160

Leu Ser Asp Pro Gly Val Gly Ala Ile Val Leu Pro Phe Thr Phe Arg
165 170 175

Trp Thr Lys Ser Phe Thr Leu Ile Leu Gln Ala Leu Asp Met Tyr Asn
180 185 190

Thr Ser Tyr Pro Asp Ala Glu Arg Leu Ile Glu Glu Thr Ser Tyr Ser
195 200 205

Gly Val Ile Leu Pro Ser Pro Glu Trp Lys Thr Leu Asp His Ile Gly
210 215 220

Arg Asn Ala Arg Ile Thr Tyr Arg Val Arg Val Gln Cys Ala Val Thr
225 230 235 240

Tyr Tyr Asn Thr Thr Cys Thr Thr Phe Cys Arg Pro Arg Asp Asp Gln
245 250 255

Phe Gly His Tyr Ala Cys Gly Ser Glu Gly Gln Lys Leu Cys Leu Asn
260 265 270

Gly Trp Gln Gly Val Asn Cys Glu Glu Ala Ile Cys Lys Ala Gly Cys
275 280 285

Asp Pro Val His Gly Lys Cys Asp Arg Pro Gly Glu Cys Glu Cys Arg
290 295 300

Pro Gly Trp Arg Gly Pro Leu Cys Asn Glu Cys Met Val Tyr Pro Gly
305 310 315 320

Cys Lys His Gly Ser Cys Asn Gly Ser Ala Trp Lys Cys Val Cys Asp
325 330 335

Thr Asn Trp Gly Gly Ile Leu Cys Asp Gln Asp Leu Asn Phe Cys Gly
340 345 350

Thr His Glu Pro Cys Lys His Gly Gly Thr Cys Glu Asn Thr Ala Pro
355 360 365

Asp Lys Tyr Arg Cys Thr Cys Ala Glu Gly Leu Ser Gly Glu Gln Cys
370 375 380

Glu Ile Val Glu His Pro Cys Ala Thr Arg Pro Cys Arg Asn Gly Gly
385 390 395 400

Thr Cys Thr Leu Lys Thr Ser Asn Arg Thr Gln Ala Gln Val Tyr Arg
405 410 415

Thr Ser His Gly Arg Ser Asn Met Gly Arg Pro Val Arg Arg Ser Ser

```
                420                 425                 430

Ser Met Arg Ser Leu Asp His Leu Arg Pro Glu Gly Gln Ala Leu Asn
        435                 440                 445

Gly Ser Ser Ser Ser Gly Leu Val Ser Leu Gly Ser Leu Gln Leu Gln
    450                 455                 460

Gln Gln Leu Ala Pro Asp Phe Thr Cys Asp Cys Ala Ala Gly Trp Thr
465                 470                 475                 480

Gly Pro Thr Cys Glu Ile Asn Ile Asp Glu Cys Ala Gly Gly Pro Cys
                485                 490                 495

Glu His Gly Gly Thr Cys Ile Asp Leu Ile Gly Gly Phe Arg Cys Glu
            500                 505                 510

Cys Pro Pro Glu Trp His Gly Asp Val Cys Gln Val Asp Val Asn Glu
        515                 520                 525

Cys Glu Ala Pro His Ser Ala Gly Ile Ala Ala Asn Ala Leu Leu Thr
    530                 535                 540

Thr Thr Ala Thr Ala Ile Ile Gly Ser Asn Leu Ser Ser Thr Ala Leu
545                 550                 555                 560

Leu Ala Ala Leu Thr Ser Ala Val Ala Ser Thr Ser Leu Ala Ile Gly
                565                 570                 575

Pro Cys Ile Asn Ala Lys Glu Cys Arg Asn Gln Pro Gly Ser Phe Ala
            580                 585                 590

Cys Ile Cys Lys Glu Gly Trp Gly Gly Val Thr Cys Ala Glu Asn Leu
        595                 600                 605

Asp Asp Cys Val Gly Gln Cys Arg Asn Gly Ala Thr Cys Ile Asp Leu
    610                 615                 620

Val Asn Asp Tyr Arg Cys Ala Cys Ala Ser Gly Phe Thr Gly Arg Asp
625                 630                 635                 640

Cys Glu Thr Asp Ile Asp Glu Cys Ala Thr Ser Pro Cys Arg Asn Gly
                645                 650                 655

Gly Glu Cys Val Asp Met Val Gly Lys Phe Asn Cys Ile Cys Pro Leu
            660                 665                 670

Gly Tyr Ser Gly Ser Leu Cys Glu Glu Ala Lys Glu Asn Cys Thr Pro
        675                 680                 685

Ser Pro Cys Leu Glu Gly His Cys Leu Asn Thr Pro Glu Gly Tyr Tyr
    690                 695                 700

Cys His Cys Pro Pro Asp Arg Ala Gly Lys His Cys Glu Gln Leu Arg
705                 710                 715                 720

Pro Leu Cys Ser Gln Pro Pro Cys Asn Glu Gly Cys Phe Ala Asn Val
                725                 730                 735

Ser Leu Ala Thr Ser Ala Thr Thr Thr Thr Thr Thr Thr Thr Thr Ala
            740                 745                 750

Thr Thr Thr Arg Lys Met Ala Lys Pro Ser Gly Leu Pro Cys Ser Gly
        755                 760                 765

His Gly Ser Cys Glu Met Ser Asp Val Gly Thr Phe Cys Lys Cys His
    770                 775                 780
```

Val Gly His Thr Gly Thr Phe Cys Glu His Asn Leu Asn Glu Cys Ser
785 790 795 800

Pro Asn Pro Cys Arg Asn Gly Gly Ile Cys Leu Asp Gly Asp Gly Asp
805 810 815

Ph Thr Cys Glu Cys Met Ser Gly Trp Thr Gly Lys Arg Cys Ser Glu
820 825 830

Arg Ala Thr Gly Cys Tyr Ala Gly Gln Cys Gln Asn Gly Gly Thr Cys
835 840 845

Met Pro Gly Ala Pro Asp Lys Ala Leu Gln Pro His Cys Arg Cys Ala
850 855 860

Pro Gly Trp Thr Gly Leu Phe Cys Ala Glu Ala Ile Asp Gln Cys Arg
865 870 875 880

Gly Gln Pro Cys His Asn Gly Gly Thr Cys Glu Ser Gly Ala Gly Trp
885 890 895

Phe Arg Cys Val Cys Ala Gln Gly Phe Ser Gly Pro Asp Cys Arg Ile
900 905 910

Asn Val Asn Glu Cys Ser Pro Gln Pro Cys Gln Gly Gly Ala Thr Cys
915 920 925

Ile Asp Gly Ile Gly Gly Tyr Ser Cys Ile Cys Pro Pro Gly Arg His
930 935 940

Gly Leu Arg Cys Glu Ile Leu Leu Ser Asp Pro Lys Ser Ala Cys Gln
945 950 955 960

Asn Ala Ser Asn Thr Ile Ser Pro Tyr Thr Ala Leu Asn Arg Ser Gln
965 970 975

Asn Trp Leu Asp Ile Ala Leu Thr Gly Arg Thr Glu Asp Asp Glu Asn
980 985 990

Cys Asn Ala Cys Val Cys Glu Asn Gly Thr Ser Arg Cys Thr Asn Leu
995 1000 1005

Trp Cys Gly Leu Pro Asn Cys Tyr Lys Val Asp Pro Leu Ser Lys Ser
1010 1015 1020

Ser Asn Leu Ser Gly Val Cys Lys Gln His Glu Val Cys Val Pro Ala
1025 1030 1035 1040

Leu Ser Glu Thr Cys Leu Ser Ser Pro Cys Asn Val Arg Gly Asp Cys
1045 1050 1055

Arg Ala Leu Glu Pro Ser Arg Arg Val Ala Pro Pro Arg Leu Pro Ala
1060 1065 1070

Lys Ser Ser Cys Trp Pro Asn Gln Ala Val Val Asn Glu Asn Cys Ala
1075 1080 1085

Arg Leu Thr Ile Leu Leu Ala Leu Glu Arg Val Gly Lys Gly Ala Ser
1090 1095 1100

Val Glu Gly Leu Cys Ser Leu Val Arg Val Leu Leu Ala Ala Gln Leu
1105 1110 1115 1120

Ile Lys Lys Pro Ala Ser Thr Phe Gly Gln Asp Pro Gly Met Leu Met
1125 1130 1135

Val Leu Cys Asp Leu Lys Thr Gly Thr Asn Asp Thr Val Glu Leu Thr

1140 1145 1150

Val Ser Ser Ser Lys Leu Asn Asp Pro Gln Leu Pro Val Ala Val Gly
1155 1160 1165

Leu Leu Gly Glu Leu Leu Ser Ser Arg Gln Leu Asn Gly Ile Gln Arg
1170 1175 1180

Arg Lys Glu Leu Glu Leu Gln His Ala Lys Leu Ala Ala Leu Thr Ser
1185 1190 1195 1200

Ile Val Glu Val Lys Leu Glu Thr Ala Arg Val Ala Asp Gly Ser Gly
1205 1210 1215

His Ser Leu Leu Ile Gly Val Leu Cys Gly Val Phe Ile Val Leu Val
1220 1225 1230

Gly Phe Ser Val Phe Ile Ser Leu Tyr Trp Lys Gln Arg Leu Ala Tyr
1235 1240 1245

Arg Thr Ser Ser Gly Met Asn Leu Thr Pro Ser Leu Asp Ala Leu Arg
1250 1255 1260

His Glu Glu Glu Lys Ser Asn Asn Leu Gln Asn Glu Glu Asn Leu Arg
1265 1270 1275 1280

Arg Tyr Thr Asn Pro Leu Lys Gly Ser Thr Ser Ser Leu Arg Ala Ala
1285 1290 1295

Thr Gly Met Glu Leu Ser Leu Asn Pro Ala Pro Glu Leu Ala Ala Ser
1300 1305 1310

Ala Ala Ser Ser Ser Ala Leu His Arg Ser Gln Pro Leu Phe Pro Pro
1315 1320 1325

Cys Asp Phe Glu Arg Glu Leu Asp Ser Ser Thr Gly Leu Lys Gln Ala
1330 1335 1340

His Lys Arg Ser Ser Gln Ile Leu Leu His Lys Thr Gln Asn Ser Asp
1345 1350 1355 1360

Met Arg Lys Asn Thr Val Gly Ser Leu Asp Ser Pro Arg Lys Asp Phe
1365 1370 1375

Gly Lys Arg Ser Ile Asn Cys Lys Ser Met Pro Pro Ser Ser Gly Asp
1380 1385 1390

Glu Gly Ser Asp Val Leu Ala Thr Thr Val Met Val
1395 1400

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 3
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:

(A) NAME/KEY: modified_base
(B) LOCATION: 12
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 18
(D) OTHER INFORMATION: /mod_base= i

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGNYTTTGCY TNAARSANTA YCA 23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /label= A
/note= "X=histidine or glutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Leu Cys Cys Lys Xaa Tyr Gln
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 3
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 9
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 12
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 15
(D) OTHER INFORMATION: /mod_base= i

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCNATGCANG TNCCNCCRTT 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asn Gly Gly Thr Cys Ile Asp
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 163 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..163

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
G TCC CGC GTC ACT GCC GGG GGA CCC TGC AGC TTC GGC TCA GGG TCT          46
  Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly Ser Gly Ser
    1               5                  10                  15

ACG CCT GTC ATC GGG GGT AAC ACC TTC AAT CTC AAG GCC AGC CGT GGC        94
Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala Ser Arg Gly
                20                  25                  30

AAC GAC CGT AAT CGC ATC GTA CTG CCT TTC AGT TTC ACC TGG CCG AGG       142
Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Thr Trp Pro Arg
            35                  40                  45

TCC TAC ACT TTG CTG GTG GAG                                          163
Ser Tyr Thr Leu Leu Val Glu
        50
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly Ser Gly Ser Thr
 1               5                  10                  15

Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala Ser Arg Gly Asn
            20                  25                  30

Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Thr Trp Pro Arg Ser
        35                  40                  45

Tyr Thr Leu Leu Val Glu
    50
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..135

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
TCT TCT AAC GTC TGT GGT CCC CAT GGC AAG TGC AAG AGC CAG TCG GCA      48
Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser Gln Ser Ala
 1               5                  10                  15

GGC AAA TTC ACC TGT GAC TGT AAC AAA GGC TTC ACC GGC ACC TAC TGC      96
Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly Thr Tyr Cys
            20                  25                  30

CAT GAA AAT ATC AAC GAC TGC GAG AGC AAC CCC TGT AAA                  135
His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Lys
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser Gln Ser Ala
 1               5                  10                  15

Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly Thr Tyr Cys
            20                  25                  30

His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Lys
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 3
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 6
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 12
(D) OTHER INFORMATION: /mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 18
(D) OTHER INFORMATION: /mod_base= i

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGNYTNTGCY TNAARSANTA YCA 23

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /label= A
/note= "X=glutamic acid or histidine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Arg Leu Cys Leu Lys Xaa Tyr Gln
1 5

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 86-87, lines 1-40 of the description '

A. IDENTIFICATION OF DEPOSIT '

Further deposits are identified on an additional sheet '

Name of depositary institution '

American Type Culture Collection

Address of depositary institution (including postal code and country) '

12301 Parklawn Drive
Rockville, MD 20852
US

Date of deposit ' February 28, 1995 Accession Number ' 97068

B. ADDITIONAL INDICATIONS ' (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ' (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS ' (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later ' (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the International application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau "

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

Accession No.	Date of Deposit
	March 5, 1996
	March 5, 1996

WHAT IS CLAIMED IS:

1. A purified vertebrate Serrate protein.

2. The protein of claim 1 which is a human protein.

3. The protein of claim 1 which is a mammalian protein.

4. The protein of claim 2 which comprises the amino acid sequence substantially as set forth in amino acid numbers 30 - 1218 of SEQ ID NO:2.

5. The protein of claim 2 which comprises the amino acid sequence substantially as set forth in amino acid numbers 1 - 1257 of SEQ ID NO:4.

6. A purified human protein encoded by a nucleic acid hybridizable to plasmid SerFL or the *Serrate* sequence therein as deposited with the ATCC and assigned accession number 68876.

7. The protein of claim 2 which is encoded by plasmid pBS39 as deposited with the ATCC and assigned accession number 97068.

8. The protein of claim 2 which comprises the Serrate amino acid sequence encoded by plasmid pBS15 as deposited with the ATCC and assigned accession number _____.

9. The protein of claim 2 which comprises the Serrate amino acid sequence encoded by plasmid pBS3-2 as deposited with the ATCC and assigned accession number _____.

10. A purified fragment of the protein of claim 1, which is able to display one or more functional activities of a Serrate protein.

11. A purified fragment of the protein of claim 2, which is able to display one or more functional activities of a human or *D. melanogaster* Serrate protein.

12. A purified fragment of the protein of claim 2 or 7, which is able to be bound by an antibody directed against a human Serrate protein.

13. A molecule comprising the fragment of claim 10.

14. A purified fragment of a vertebrate Serrate protein comprising a domain of the protein selected from the group consisting of the extracellular domain, DSL domain, epidermal growth factor-like repeat domain, cysteine-rich domain, transmembrane domain, and intracellular domain.

15. A purified fragment of a vertebrate Serrate protein comprising the DSL domain of the protein.

16. A purified fragment of a vertebrate Serrate protein comprising an epidermal growth factor-homologous repeat of the protein.

17. The fragment of claim 14 in which the Serrate protein is a human Serrate protein.

18. A purified fragment of a vertebrate Serrate protein comprising a region homologous to a Notch protein or a Delta protein, and consisting of at least ten amino acids.

19. A chimeric protein comprising a fragment of a vertebrate Serrate protein consisting of at least ten amino

acids fused via a covalent bond to an amino acid sequence of a second protein, in which the second protein is not a Serrate protein.

20. The chimeric protein of claim 19 in which the fragment of a Serrate protein is a fragment capable of being bound by an anti-Serrate antibody.

21. The chimeric protein of claim 19 in which the Serrate protein is a human protein.

22. The chimeric protein of claim 19 which is able to display one or more functional activities of a Serrate protein.

23. A purified fragment of a vertebrate Serrate protein which fragment (a) is capable of being bound by an anti-Serrate antibody; (b) lacks the transmembrane and intracellular domains of the protein; and (c) consists of at least ten amino acids of the Serrate protein.

24. A purified fragment of a vertebrate Serrate protein which fragment (a) is capable of being bound by an anti-Serrate antibody; (b) lacks the extracellular domain of the protein; and (c) consists of at least ten amino acids of the Serrate protein.

25. A purified fragment of a vertebrate Serrate protein which is able to bind to a Notch protein.

26. The fragment of claim 25, which lacks the epidermal growth factor-like repeats of the Serrate protein.

27. The fragment of claim 23, 24, 25 or 26 in which the Serrate protein is a human Serrate protein.

28. The fragment of claim 29, which is a fragment of SEQ ID NO:2 or SEQ ID NO:4.

29. A molecule comprising the fragment of claim 25.

30. An antibody which is capable of binding the Serrate protein of claim 1 and which does not bind a *Drosophila* Serrate protein.

31. An antibody which is capable of binding the Serrate protein of claim 2 and which does not bind a *Drosophila* Serrate protein.

32. The antibody of claim 30 which is monoclonal.

33. A molecule comprising a fragment of the antibody of claim 32, which fragment is capable of binding a vertebrate Serrate protein.

34. An isolated nucleic acid comprising a nucleotide sequence encoding a vertebrate Serrate protein.

35. The nucleic acid of claim 34 which is DNA.

36. An isolated nucleic acid comprising a nucleotide sequence absolutely complementary to the nucleotide sequence of claim 34.

37. An isolated nucleic acid comprising a nucleotide sequence encoding the Serrate protein of claim 2.

38. An isolated nucleic acid comprising the *Serrate* coding sequence contained in plasmid pBS39 as deposited with the ATCC and assigned accession number 97068.

39. An isolated human nucleic acid hybridizable to plasmid SerFL or the *Serrate* sequence ther in as deposited with the ATCC and assigned accession number 68876.

40. An isolated nucleic acid comprising the *Serrate* coding sequence contained in plasmid pBS3-2 as deposited with the ATCC and assigned accession number _____.

41. An isolated nucleic acid comprising the *Serrate* coding sequence contained in plasmid pBS15 as deposited with the ATCC and assigned accession number _____.

42. An isolated nucleic acid comprising a nucleotide sequence encoding a protein, said protein comprising amino acid numbers 1 - 1257 of SEQ ID NO:4.

43. An isolated nucleic acid comprising a fragment of a vertebrate *Serrate* gene consisting of at least 8 nucleotides.

44. An isolated nucleic acid comprising a nucleotide sequence encoding the fragment of claim 14, 15, 16 or 25.

45. The nucleic acid of claim 44 in which the fragment is a fragment of a human Serrate protein.

46. An isolated nucleic acid comprising a nucleotide sequence encoding the fragment of claim 12.

47. An isolated nucleic acid comprising a nucleotide sequence encoding a protein, said protein comprising amino acid numbers 30 - 1218 of SEQ ID NO:2.

48. An isolated nucleic acid comprising a nucl otide sequ nce encoding the prot in of claim 21.

49. A recombinant cell containing the nucleic acid of claim 34, 37 or 43.

50. A recombinant cell containing the nucleic acid of claim 38, 40 or 41.

51. A method of producing a Serrate protein comprising growing a recombinant cell containing the nucleic acid of claim 34 or 37 such that the encoded Serrate protein is expressed by the cell, and recovering the expressed Serrate protein.

52. A method of producing a Serrate protein comprising growing a recombinant cell containing the nucleic acid of claim 38, 40 or 41 such that the encoded Serrate protein is expressed by the cell, and recovering the expressed Serrate protein.

53. A method of producing a Serrate protein comprising growing a recombinant cell containing the nucleic acid of claim 45 such that the encoded protein is expressed by the cell, and recovering the expressed protein.

54. A method of producing a protein comprising a fragment of a Serrate protein, which method comprises growing a recombinant cell containing the nucleic acid of claim 46 such that the encoded protein is expressed by the cell, and recovering the expressed protein.

55. The product of the process of claim 51.

56. The product of the process of claim 52.

57. The product of the process of claim 53.

58. The product of the process of claim 54.

59. A pharmaceutical composition comprising a therapeutically effective amount of a vertebrate Serrate protein; and a pharmaceutically acceptable carrier.

60. The composition of claim 59 in which the Serrate protein is a human Serrate protein.

61. A pharmaceutical composition comprising a therapeutically effective amount of the fragment of claim 14, 15, 16 or 25; and a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising a therapeutically effective amount of the fragment of claim 12; and a pharmaceutically acceptable carrier.

63. A pharmaceutical composition comprising a therapeutically effective amount of a molecule comprising a fragment of a vertebrate Serrate protein, which derivative or analog is characterized by the ability to bind to a Notch protein or to a molecule comprising the epidermal growth factor-like repeats 11 and 12 of a Notch protein.

64. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 34, 36 or 37; and a pharmaceutically acceptable carrier.

65. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 44; and a pharmaceutically acceptable carrier.

66. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 46; and a pharmaceutically acceptable carrier.

67. A pharmaceutical composition comprising a therapeutically effective amount of the antibody of claim 30; and a pharmaceutically acceptable carrier.

68. A pharmaceutical composition comprising a therapeutically effective amount of a fragment or derivative of the antibody of claim 30 containing the binding domain of the antibody; and a pharmaceutically acceptable carrier.

69. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a vertebrate Serrate protein or derivative thereof which is able to bind to a Notch protein.

70. The method according to claim 69 in which the disease or disorder is a malignancy characterized by increased Notch activity or increased expression of a Notch protein or of a Notch derivative capable of being bound by an anti-Notch antibody, relative to said Notch activity or expression in an analogous non-malignant sample.

71. The method according to claim 69 in which the disease or disorder is selected from the group consisting of cervical cancer, breast cancer, colon cancer, melanoma, seminoma, and lung cancer.

72. The method according to claim 69 in which the subject is a human.

73. The method according to claim 69 in which the Serrate protein is a human Serrate protein.

74. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule, in which the molecule is an oligonucleotide which (a) comprises ten nucleotides; (b) comprises a sequence absolutely complementary to an at l ast t n nucleotid portion of an RNA

transcript specific to a vertebrate *Serrate* gene; and (c) is hybridizable to th RNA transcript.

75. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired an effective amount of the nucleic acid of claim 34, 37 or 46.

76. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired an effective amount of the antibody of claim 32.

77. The method according to claim 73 in which the disease or disorder is a disease or disorder of the central nervous system.

78. An isolated oligonucleotide comprising ten nucleotides, and comprising a sequence absolutely complementary to an at least ten nucleotide portion of an RNA transcript specific to a vertebrate *Serrate* gene, which oligonucleotide is hybridizable to the RNA transcript.

79. A pharmaceutical composition comprising the oligonucleotide of claim 78; and a pharmaceutically acceptable carrier.

80. A method of inhibiting the expression of a nucleic acid sequence encoding a Serrate protein in a cell comprising providing the cell with an effective amount of the oligonucleotide of claim 78.

81. A method of diagnosing a disease or disorder characterized by an aberrant level of Notch-Serrate protein binding activity in a patient, comprising measuring the ability of a Notch protein in a sample derived from the patient to bind to a vertebrate Serrate protein, in which an

increase or decrease in the ability of the Notch protein to bind to the Serrate protein, relative to the ability found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

82. A method of diagnosing a disease or disorder characterized by an aberrant level of Serrate protein in a patient, comprising measuring the levels of a vertebrate Serrate protein in a sample derived from the patient, in which an increase or decrease in the levels of the Serrate protein, relative to the levels of the Serrate protein found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

```
        10          20          30          40          50          60
GAATTCCCCT CCCCCCTTTT TCCATGCAGC TGATCTAAAA GGGAATAAAA GGCTGCGCAT
        70          80          90         100         110         120
AATCATAATA ATAAAAGAAG GGGAGCGCGA GAGAAGGAAA GAAAGCCGGG AGGTGGAAGA
       130         140         150         160         170         180
GGAGGGGGAG CGTCTCAAAG AAGCGATCAG AATAATAAAA GGAGGCCGGG CTCTTTGCCT
       190         200         210         220         230         240
TCTGGAAGGG GCCGCTCTTG AAAGGGCTTT TGAAAAGTGG TGTTGTTTTC CAGTCGTGCA
       250         260         270         280         290         300
TGCTCCAATC GGCGGAGTAT ATTAGAGCCG GGACGCGGCC GCAGGGGCAG CGGCGACGGC
       310         320         330         340         350         360
AGCACCGGCG GCAGCACCAG CGCGAACAGC AGCGGCGGCG TCCCGAGTGC CCGCGGCGGC
       370         380         390         400         410         420
GCGCGCAGCG ATGCGTTCCC CACGGACACG CGGCCGGTCC GGGCGCCCCC TAAGCCTCCT
             M   R   S   P   R   T   R   G   R   S   G   R   P   L   S   L   L>
       430         440         450         460         470         480
GCTCGCCCTG CTCTGTGCCC TGCGAGCCAA GGTGTGTGGG GCCTCGGGTC AGTTCGAGTT
  L   A   L   L   C   A   L   R   A   K   V   C   G   A   S   G   Q   F   E   L>
       490         500         510         520         530         540
GGAGATCCTG TCCATGCAGA ACGTGAACGG GGAGCTGCAG AACGGGAACT GCTGCGGCGG
  E   I   L   S   M   Q   N   V   N   G   E   L   Q   N   G   N   C   C   G   G>
       550         560         570         580         590         600
CGCCCGGAAC CCGGGAGACC GCAAGTGCAC CCGCGACGAG TGTGACACAT ACTTCAAAGT
  A   R   N   P   G   D   R   K   C   T   R   D   E   C   D   T   Y   F   K   V>
       610         620         630         640         650         660
GTGCCTCAAG GAGTATCAGT CCCGCGTCAC GGCCGGGGGG CCCTGCAGCT TCGGCTCAGG
  C   L   K   E   Y   Q   S   R   V   T   A   G   G   P   C   S   F   G   S   G>
       670         680         690         700         710         720
GTCCACGCCT GTCATCGGGG GCAACACCTT CAACCTCAAG GCCAGCCGCG GCAACGACCC
  S   T   P   V   I   G   G   N   T   F   N   L   K   A   S   P   G   N   D   P>
       730         740         750         760         770         780
GAACCGCATC GTGCTGCCTT TCAGTTTCGC CTGGCCGAGG TCCTATACGT TGCTTGTGGA
  N   R   I   V   L   P   F   S   F   A   W   P   R   S   Y   T   L   L   V   E>
       790         800         810         820         830         840
GGCGTGGGAT TCCAGTAATG ACACCGTTCA ACCTGACAGT ATTATTGAAA AGGCTTCTCA
  A   W   D   S   S   N   D   T   V   Q   P   D   S   I   I   E   K   A   S   H>
       850         860         870         880         890         900
CTCGGGCATG ATCAACCCCA GCCGGCAGTG GCAGACGCTG AAGCAGAACA CGGGCGTTGC
  S   G   M   I   N   P   S   R   Q   W   Q   T   L   K   Q   N   T   G   V   A>
```

FIG. 1A

```
      910         920         930         940         950         960
CCACTTTGAG TATCAGATCC GCGTGACCTG TGATGACTAC TACTATGGCT TTGGCTGTAA
  H  F  E   Y  Q  I   R  V  T  C   D  D  Y   Y  Y  G   F  G  C  N>
      970         980         990        1000        1010        1020
TAAGTTCTGC CGCCCCAGAG ATGACTTCTT TGGACACTAT GCCTGTGACC AGAATGGCAA
  K  F  C   R  P  R   D  D  F  F   G  H  Y   A  C  D   Q  N  G  N>
     1030        1040        1050        1060        1070        1080
CAAAACTTGC ATGGAAGGCT GGATGGGCCC CGAATGTAAC AGAGCTATTT GCCGACAAGG
  K  T  C   M  E  G   W  M  G  P   E  C  N   R  A  I   C  R  Q  G>
     1090        1100        1110        1120        1130        1140
CTGCAGTCCT AAGCATGGGT CTTGCAAACT CCCAGGTGAC TGCAGGTGCC AGTACGGCTG
  C  S  P   K  H  G   S  C  K  L   P  G  D   C  R  C   Q  Y  G  W>
     1150        1160        1170        1180        1190        1200
GCAAGGCCTG TACTGTGATA AGTGCATCCC ACACCCGGGA TGCGTCCACG GCATCTGTAA
  Q  G  L   Y  C  D   K  C  I  P   H  P  G   C  V  H   G  I  C  N>
     1210        1220        1230        1240        1250        1260
TGAGCCCTGG CAGTGCCTCT GTGAGACCAA CTGGGGCGGC CAGCTCTGTG ACAAAGATCT
  E  P  W   Q  C  L   C  E  T  N   W  G  G   Q  L  C   D  K  D  L>
     1270        1280        1290        1300        1310        1320
CAATTACTGT GGGACTCATC AGCCGTGTCT CAACGGGGGA ACTTGTAGCA ACACAGGCCC
  N  Y  C   G  T  H   Q  P  C  L   N  G  G   T  C  S   N  T  G  P>
     1330        1340        1350        1360        1370        1380
TGACAAATAT CAGTGTTCCT GCCCTGAGGG GTATTCAGGA CCCAACTGTG AAATTGCTGA
  D  K  Y   Q  C  S   C  P  E  G   Y  S  G   P  N  C   E  I  A  E>
     1390        1400        1410        1420        1430        1440
GCACGCCTGC CTCTCTGATC CCTGTCACAA CAGAGGCAGC TGTAAGGAGA CCTCCCTGGG
  H  A  C   L  S  D   P  C  H  N   R  G  S   C  K  E   T  S  L  G>
     1450        1460        1470        1480        1490        1500
CTTTGAGTGT GAGTGTTCCC CAGGCTGGAC CGGCCCCACA TGCTCTACAA ACATTGATGA
  F  E  C   E  C  S   P  G  W  T   G  P  T   C  S  T   N  I  D  D>
     1510        1520        1530        1540        1550        1560
CTGTTCTCCT AATAACTGTT CCCACGGGGG CACCTGCCAG GACCTGGTTA ACGGATTTAA
  C  S  P   N  N  C   S  H  G  G   T  C  Q   D  L  V   N  G  F  K>
     1570        1580        1590        1600        1610        1620
GTGTGTGTGC CCCCCACAGT GGACTGGGAA AACGTGCCAG TTAGATGCAA ATGAATGTGA
  C  V  C   P  P  Q   W  T  G  K   T  C  Q   L  D  A   N  E  C  E>
     1630        1640        1650        1660        1670        1680
GGCCAAACCT TGTGTAAACG CCAAATCCTG TAAGAATCTC ATTGCCAGCT ACTACTGCGA
  A  K  P   C  V  N   A  K  S  C   K  N  L   I  A  S   Y  Y  C  D>
```

FIG. 1B

```
      1690        1700        1710        1720        1730        1740
CTGTCTTCCC GGCTGGATGG GTCAGAATTG TGACATAAAT ATTAATGACT GCCTTGGCCA
  C  L  P   G  W  M   G  Q  N  C   D  I  N   I  N  D   C  L  G  Q>
      1750        1760        1770        1780        1790        1800
GTGTCAGAAT GACGCCTCCT GTCGGGATTT GGTTAATGGT TATCGCTGTA TCTGTCCACC
  C  Q  N   D  A  S   C  R  D  L   V  N  G   Y  R  C   I  C  P  P>
      1810        1820        1830        1840        1850        1860
TGGCTATGCA GGCGATCACT GTGAGAGAGA CATCGATGAA TGTGCCAGCA ACCCCTGTTT
  G  Y  A   G  D  H   C  E  R  D   I  D  E   C  A  S   N  P  C  L>
      1870        1880        1890        1900        1910        1920
GAATGGGGGT CACTGTCAGA ATGAAATCAA CAGATTCCAG TGTCTGTGTC CCACTGGTTT
  N  G  G   H  C  Q   N  E  I  N   R  F  Q   C  L  C   P  T  G  F>
      1930        1940        1950        1960        1970        1980
CTCTGGAAAC CTCTGTCAGC TGGACATCGA TTATTGTGAG CCTAATCCCT GCCAGAACGG
  S  G  N   L  C  Q   L  D  I  D   Y  C  E   P  N  P   C  Q  N  G>
      1990        2000        2010        2020        2030        2040
TGCCCAGTGC TACAACCGTG CCAGTGACTA TTTCTGCAAG TGCCCCGAGG ACTATGAGGG
  A  Q  C   Y  N  R   A  S  D  Y   F  C  K   C  P  E   D  Y  E  G>
      2050        2060        2070        2080        2090        2100
CAAGAACTGC TCACACCTGA AAGACCACTG CCGCACGACC CCCTGTGAAG TGATTGACAG
  K  N  C   S  H  L   K  D  H  C   R  T  T   P  C  E   V  I  D  S>
      2110        2120        2130        2140        2150        2160
CTGCACAGTG GCCATGGCTT CCAACGACAC ACCTGAAGGG GTGCGGTATA TTTCCTCCAA
  C  T  V   A  M  A   S  N  D  T   P  E  G   V  R  Y   I  S  S  N>
      2170        2180        2190        2200        2210        2220
CGTCTGTGGT CCTCACGGGA AGTGCAAGAG TCAGTCGGGA GGCAAATTCA CCTGTGACTG
  V  C  G   P  H  G   K  C  K  S   Q  S  G   G  K  F   T  C  D  C>
      2230        2240        2250        2260        2270        2280
TAACAAAGGC TTCACGGGAA CATACTGCCA TGAAAATATT AATGACTGTG AGAGCAACCC
  N  K  G   F  T  G   T  Y  C  H   E  N  I   N  D  C   E  S  N  P>
      2290        2300        2310        2320        2330        2340
TTGTAGAAAC GGTGGCACTT GCATCGATGG TGTCAACTCC TACAAGTGCA TCTGTAGTGA
  C  R  N   G  G  T   C  I  D  G   V  N  S   Y  K  C   I  C  S  D>
      2350        2360        2370        2380        2390        2400
CGGCTGGGAG GGGGCCTACT GTGAAACCAA TATTAATGAC TGCAGCCAGA ACCCCTGCCA
  G  W  E   G  A  Y   C  E  T  N   I  N  D   C  S  Q   N  P  C  H>
      2410        2420        2430        2440        2450        2460
CAATGGGGGC ACGTGTCGCG ACCTGGTCAA TGACTTCTAC TGTGACTGTA AAAATGGGTG
  N  G  G   T  C  R   D  L  V  N   D  F  Y   C  D  C   K  N  G  W>
```

FIG. 1C

```
      2470        2480        2490        2500        2510        2520
GAAAGGAAAG ACCTGCCACT CACGTGACAG TCAGTGTGAT GAGGCCACGT GCAACAACGG
  K  G  K    T  C  H    S  R  D  S    Q  C  D    E  A  T    C  N  N  G>
      2530        2540        2550        2560        2570        2580
TGGCACCTGC TATGATGAGG GGGATGCTTT TAAGTGCATG TGTCCTGGCG GCTGGGAAGG
  G  T  C    Y  D  E    G  D  A  F    K  C  M    C  P  G    G  W  E  G>
      2590        2600        2610        2620        2630        2640
AACAACCTGT AACATAGCCC GAAACAGTAG CTGCCTGCCC AACCCCTGCC ATAATGGGGG
  T  T  C    N  I  A    R  N  S  S    C  L  P    N  P  C    H  N  G  G>
      2650        2660        2670        2680        2690        2700
CACATGTGTG GTCAACGGCG AGTCCTTTAC GTGCGTCTGC AAGGAAGGCT GGGAGGGGCC
  T  C  V    V  N  G    E  S  F  T    C  V  C    K  E  G    W  E  G  P>
      2710        2720        2730        2740        2750        2760
CATCTGTGCT CAGAATACCA ATGACTGCAG CCCTCATCCC TGTTACAACA GCGGCACCTG
  I  C  A    Q  N  T    N  D  C  S    P  H  P    C  Y  N    S  G  T  C>
      2770        2780        2790        2800        2810        2820
TGTGGATGGA GACAACTGGT ACCGGTGCGA ATGTGCCCCG GGTTTTGCTG GGCCCGACTG
  V  D  G    D  N  W    Y  R  C  E    C  A  P    G  F  A    G  P  D  C>
      2830        2840        2850        2860        2870        2880
CAGAATAAAC ATCAATGAAT GCCAGTCTTC ACCTTGTGCC TTTGGAGCGA CCTGTGTGGA
  R  I  N    I  N  E    C  Q  S  S    P  C  A    F  G  A    T  C  V  D>
      2890        2900        2910        2920        2930        2940
TGAGATCAAT GGCTACCGGT GTGTCTGCCC TCCAGGGCAC AGTGGTGCCA AGTGCCAGGA
  E  I  N    G  Y  R    C  V  C  P    P  G  H    S  G  A    K  C  Q  E>
      2950        2960        2970        2980        2990        3000
AGTTTCAGGG AGACCTTGCA TCACCATGGG GAGTGTGATA CCAGATGGGG CCAAATGGGA
  V  S  G    R  P  C    I  T  M  G    S  V  I    P  D  G    A  K  W  D>
      3010        3020        3030        3040        3050        3060
TGATGACTGT AATACCTGCC AGTGCCTGAA TGGACGGATC GCCTGCTCAA AGGTCTGGTG
  D  D  C    N  T  C    Q  C  L  N    G  R  I    A  C  S    K  V  W  C>
      3070        3080        3090        3100        3110        3120
TGGCCCTCGA CCTTGCCTGC TCCACAAAGG GCACAGCGAG TGCCCCAGCG GGCAGAGCTG
  G  P  R    P  C  L    L  H  K  G    H  S  E    C  P  S    G  Q  S  C>
      3130        3140        3150        3160        3170        3180
CATCCCCATC CTGGACGACC AGTGCTTCGT CCACCCCTGC ACTGGTGTGG GCGAGTGTCG
  I  P  I    L  D  D    Q  C  F  V    H  P  C    T  G  V    G  E  C  R>
      3190        3200        3210        3220        3230        3240
GTCTTCCAGT CTCCAGCCGG TGAAGACAAA GTGCACCTCT GACTCCTATT ACCAGGATAA
  S  S  S    L  Q  P    V  K  T  K    C  T  S    D  S  Y    Y  Q  D  N>
      3250        3260        3270        3280        3290        3300
CTGTGCGAAC ATCACATTTA CCTTTAACAA GGAGATGATG TCACCAGGTC TTACTACGGA
  C  A  N    I  T  F    T  F  N  K    E  M  M    S  P  G    L  T  T  E>
```

FIG. 1D

```
      3310        3320        3330        3340        3350        3360
GCACATTTGC AGTGAATTGA GGAATTTGAA TATTTTGAAG AATGTTTCCG CTGAATATTC
  H   I   C   S   E   L   R   N   L   N   I   L   K   N   V   S   A   E   Y   S>
      3370        3380        3390        3400        3410        3420
AATCTACATC GCTTGCGAGC CTTCCCCTTC AGCGAACAAT GAAATACATG TGGCCATTTC
  I   Y   I   A   C   E   P   S   P   S   A   N   N   E   I   H   V   A   I   S>
      3430        3440        3450        3460        3470        3480
TGCTGAAGAT ATACGGGATG ATGGGAACCC GATCAAGGAA ATCACTGACA AAATAATCGA
  A   E   D   I   R   D   D   G   N   P   I   K   E   I   T   D   K   I   I   D>
      3490        3500        3510        3520        3530        3540
TCTTGTTACT AAACGTGATG GAAACAGCTC GCTGATTGCT GCCGTTGAAG AAGTAAGAGT
  L   V   T   K   R   D   G   N   S   S   L   I   A   A   V   E   E   V   R   V>
      3550        3560        3570        3580        3590        3600
TCAGAGGCGG CCTCTGAAGA ACAGAACAGA TTTCCTTGTT CCCTTGCTGA GCTCTGTCTT
  Q   R   R   P   L   K   N   R   T   D   F   L   V   P   L   L   S   S   V   L>
      3610        3620        3630        3640        3650        3660
AACTGTGGCT TGGATCTGTT GCTTGGTGAC GGCCTTCTAC TGGTGCCTGC GGAAGCGGCG
  T   V   A   W   I   C   C   L   V   T   A   F   Y   W   C   L   R   K   R   R>
      3670        3680        3690        3700        3710        3720
GAAGCCGGGC AGCCACACAC ACTCAGCCTC TGAGGACAAC ACCACCAACA ACGTGCGGGA
  K   P   G   S   H   T   H   S   A   S   E   D   N   T   T   N   N   V   R   E>
      3730        3740        3750        3760        3770        3780
GCAGCTGAAC CAGATCAAAA ACCCCATTGA GAAACATGGG GCCAACACGG TCCCCATCAA
  Q   L   N   Q   I   K   N   P   I   E   K   H   G   A   N   T   V   P   I   K>
      3790        3800        3810        3820        3830        3840
GGATTACGAG AACAAGAACT CCAAAATGTC TAAAATAAGG ACACACAATT CTGAAGTAGA
  D   Y   E   N   K   N   S   K   M   S   K   I   R   T   H   N   S   E   V   E>
      3850        3860        3870        3880        3890        3900
AGAGGACGAC ATGGACAAAC ACCAGCAGAA AGCCCGGTTT GCCAAGCAGC CGGCGTACAC
  E   D   D   M   D   K   H   Q   Q   K   A   R   F   A   K   Q   P   A   Y   T>
      3910        3920        3930        3940        3950        3960
GCTGGTAGAC AGAGAAGAGA AGCCCCCCAA CGGCACGCCG ACAAAACACC CAAACTGGAC
  L   V   D   R   E   E   K   P   P   N   G   T   P   T   K   H   P   N   W   T>
      3970        3980        3990        4000        4010        4020
AAACAAACAG GACAACAGAG ACTTGGAAAG TGCCCAGAGC TTAAACCGAA TGGAGTACAT
  N   K   Q   D   N   R   D   L   E   S   A   Q   S   L   N   R   M   E   Y   I>
      4030        4040        4050        4060        4070        4080
CGTATAGCAG ACCGCGGGCA CTGCCGCCGC TAGGTAGAGT CTGAGGGCTT GTAGTTCTTT
  V   >
```

FIG. 1E

```
      4090        4100        4110        4120        4130        4140
AAACTGTCGT GTCATACTCG AGTCTGAGGC CGTTGCTGAC TTAGAATCCC TGTGTTAATT
      4150        4160        4170        4180        4190        4200
TAGTTTGACA AGCTGGCTTA CACTGGCAAT GGTAGTTCTG TGGTTGGCTG GGAAATCGAG
      4210        4220        4230        4240        4250        4260
TGGCGCATCT CACAGCTATG CAAAAAGCTA GTCAACAGTA CCCCTGGTTG TGTGTCCCCT
      4270        4280        4290        4300        4310        4320
TGCAGCCGAC ACGGTCTCGG ATCAGGCTCC CAGGAGCTGC CCAGCCCCCT GGTACTTTGA
      4330        4340        4350        4360        4370        4380
GCTCCCACTT CTGCCAGATG TCTAATGGTG ATGCAGTCTT AGATCATAGT TTTATTTATA
      4390        4400        4410        4420        4430        4440
TTTATTGACT CTTGAGTTGT TTTTGTATAT TGGTTTTATG ATGACGTACA AGTAGTTCTG
      4450        4460        4470        4480        4490        4500
TATTTGAAAG TGCCTTTGCA GCTCAGAACC ACAGCAACGA TCACAAATGA CTTTATTATT
      4510        4520        4530        4540        4550        4560
TATTTTTTTT AATTGTATTT TTGTTGTTGG GGGAGGGGAG ACTTTGATGT CAGCAGTTGC
      4570        4580        4590        4600        4610        4620
TGGTAAAATG AAGAATTTAA AGAAAAAATG TCCAAAAGTA GAACTTTGTA TAGTTATGTA
      4630        4640        4650        4660        4670        4680
AATAATTCTT TTTTATTAAT CACTGTGTAT ATTTGATTTA TTAACTTAAT AATCAAGAGC
      4690        4700        4710        4720        4730        4740
CTTAAAACAT CATTCCTTTT TATTTATATG TATGTGTTTA GAATTGAAGG TTTTTGATAG
      4750        4760        4770        4780        4790        4800
CATTGTAAGC GTATGGCTTT ATTTTTTTGA ACTCTTCTCA TTACTTGTTG CCTATAAGCC
      4810        4820        4830        4840        4850        4860
AAAAAGGAAA GGGTGTTTTG AAAATAGTTT ATTTTAAAAC AATAGGATGG GCTACACGTA
      4870        4880        4890        4900        4910        4920
CATAGGTAAA TAATAGCACC GTACTGGTTA TGATGATGAA AATAACTGGA AACTTGAAAG
      4930        4940        4950        4960        4970        4980
CTTGTGGTAA TGGCAGATAA AGATGGTTCA CCTGGGAAAT TAAAACTTGA ATGGTTGTAC
      4990        5000        5010        5020        5030        5040
AGAAAAGCAC AGAGTGGAAT GCACATCAAT GACAGTAAGG GAGTTAGTTC TAGGAACAGC
      5050        5060        5070        5080        5090        5100
TCCTGAACAG TAAGATTCCC GCAATAGTCT CCGCCTCGTT CGTCTATGGT ATGCATCCCA
      5110        5120        5130        5140        5150        5160
TTCATTTTCT TCTTCTGATT ATTGTCATCT TTCCCTTTGC CAAATGGGCA GTTATTGTTT
      5170        5180        5190        5200        5210        5220
CAGGGAGAGA AGCTGCTCAT TGGCCAATCA TTCTGGTGTG CAGTGCTCCA TCGGATTCTA
      5230        5240        5250        5260        5270        5280
CATGTCCAAC AAGGCATGTC TGGATGATGC AATGTCTGTC TGACCCCCGG AATTCCGTGC
```

FIG. 1F

```
      5290        5300        5310        5320        5330        5340
AGAGACAACA TTCTAGACAG ATATACACTT TTTATTATTA ACAAACTTTG GCCACAACCT
      5350        5360        5370        5380        5390        5400
TTGATGTATA AATTGCCGGA TTTCCCCAGT CCTTTCATTG TGGCTTTGGA CAGGAGCAGG
      5410        5420        5430        5440        5450        5460
CTCACTTGTC TGCTTCAGGC TGCCTTTCTC TTGGGTTGCA CCTCAGTTCT TACTTATTTA
      5470        5480        5490        5500        5510        5520
TTTATTTTGA GTGGAGCATA GGGGCCTCTT CCAAAATGGG TAGAGCTCAG GGGCTTTCTT
      5530        5540        5550        5560        5570        5580
ATTGAAATGG TCACATGATA AAAACGGGCT GAAAAAGGAG AGTTCCAGGA GAAAAGCCCA
      5590        5600        5610        5620        5630        5640
GAAAAGGCCC CTCCTCAGAA GACAGCCTTT AAGCCTCTTG CTTACTGAAG GAAGCCCCAC
      5650        5660        5670        5680        5690        5700
CTTCTAGCAC TGAGGCCGGG TCTGATCTTC CAGAGGAGTT GGAGGAGTCC ATGAGAATGG
      5710        5720        5730        5740        5750        5760
CCACCATTCT TGCTTGCTGC TGCTGATGTT GCAGTTTTGA GAGAACAGCG GGATCCTTGT
      5770        5780        5790        5800        5810        5820
TGTCCTCTAG AGACTTGAGT CTGTCACTGA CATTTTTTCA GTTCCTTTGC TCATAGACCA
      5830        5840        5850        5860        5870        5880
TACGAGGAAT TAGTGATGTG TCAGTTGAGA GTTCACAATC TCATTGTTCA TTTAATTCAC
      5890        5900        5910        5920        5930        5940
TTTAAAGTTG TCAATTTCTG TGTGAGTAAC CTGTAAAAGA CACCTTTCCA GAAGAGTTTT
      5950        5960        5970        5980        5990        6000
GCCGTCTGTT TGAAAAAAAA ATCTTTATAA ACTTTCCTAA GTATCTGGAT TTGGATTCCT
      6010        6020        6030        6040        6050        6060
TATTTGGAGA GAAAATGTAC CCTGTCTCCA CCAAAAATAC AAAAATTAGC CAGGCTTGGT
      6070        6080        6090        6100        6110        6120
GGTGCACACC GGTAATCCCA GCAACTCTGG AGACTAAGGC AGGAAGAATC GCTTGACCCA
      6130        6140        6150        6160        6170        6180
GGAGGGTCGA GGCTACAATG AGTTGAAACC GCGCCACTGC ACTCCAGCCT GGGCGACAGT
      6190        6200        6210        6220        6230        6240
GCGAGGCCCT GTCTCAAAAA TAAAATAAAA TAAATAAATA AATTAGCCAG ATACTGTGTG
      6250        6260        6270        6280        6290        6300
CACGCCTGCA GTCCCAGCTA TTCTGGAAGC TGAGGTGGGA AGATGGTTAA GCCTGAGAGG
      6310        6320        6330        6340        6350        6360
ACAAAGCTGC AGTGAGTCAT GTTTGCATCA CTGCACTCCA GCCTGGGTGA CAGAGCAAGA
      6370        6380        6390        6400        6410        6420
CCCTGTCTAA AAAACAAAAA CAGGCCGGGT GTGGTGGCTC ATGCCTGCCA TCCCAGTGCT
      6430        6440        6450        6460
TTGGGAGGCA GAGGTTGGCA TAATCCCAGC GCTCTGGGAA TTCC
```

FIG. 1G

```
GGCCGGGGCC GGGCGGGCGG GTCGCGGGGG CAATGCGGGC GCAGGGCCGG GGGCGCCTTC  60

CCCGGCGGCT GCTGCTGCTG CTGGCGCTCT GGGTGCAGGC GGCGCGGCCC ATGGGCTATT 120

TCGAGCTGCA GCTGAGCGCG CTGCGGAACG TGAACGGGGA GCTGCTGAGC GGCGCCTGCT 180

GTGACGGCGA CGGCCGGACA ACGCGCGCGG GGGGCTGCGG CCACGACGAG TGCGACACCG 240

CTCCTTTACC CTCATCGTGG AGGCCTGGGA CTGGGACAAC GATACCACCC CGAATGAGGA 300

GCTGCTGATC GAGCGAGTGT CGCATGCCGG C ATG ATC AAC CCG GAG GAC CGC    352
                                  Met Ile Asn Pro Glu Asp Arg
                                    1               5

TGG AAG AGC CTG CAC TTC AGC GGC CAC GTG GCG CAC CTG GAG CTG CAG   400
Trp Lys Ser Leu His Phe Ser Gly His Val Ala His Leu Glu Leu Gln
        10                  15                  20

ATC CGC GTG CGC TGC GAC GAG AAC TAC TAC AGC GCC ACT TGC AAC AAG   448
Ile Arg Val Arg Cys Asp Glu Asn Tyr Tyr Ser Ala Thr Cys Asn Lys
    25                  30                  35

TTC TGC CGG CCC CGC AAT GAC TTT TTC GGC CAC TAC ACC TGC GAC CAG   496
Phe Cys Arg Pro Arg Asn Asp Phe Phe Gly His Tyr Thr Cys Asp Gln
40                  45                  50                  55

TAC GGC AAC AAG GCC TGC ATG GAC GGC TGG ATG GGC AAG GAG TGC AAG   544
Tyr Gly Asn Lys Ala Cys Met Asp Gly Trp Met Gly Lys Glu Cys Lys
                60                  65                  70

GAA GCT GTG TGT AAA CAA GGG TGT AAT TTG CTC CAC GGG GGA TGC ACC   592
Glu Ala Val Cys Lys Gln Gly Cys Asn Leu Leu His Gly Gly Cys Thr
            75                  80                  85

GTG CCT GGG GAG TGC AGG TGC AGC TAC GGC TGG CAA GGG AGG TTC TGC   640
Val Pro Gly Glu Cys Arg Cys Ser Tyr Gly Trp Gln Gly Arg Phe Cys
        90                  95                  100

GAT GAG TGT GTC CCC TAC CCC GGC TGC GTG CAT GGC AGT TGT GTG GAG   688
Asp Glu Cys Val Pro Tyr Pro Gly Cys Val His Gly Ser Cys Val Glu
    105                 110                 115

CCC TGG CAG TGC AAC TGT GAG ACC AAC TGG GGC GGC CTG CTC TGT GAC   736
Pro Trp Gln Cys Asn Cys Glu Thr Asn Trp Gly Gly Leu Leu Cys Asp
120                 125                 130                 135

AAA GAC CTG AAC TAC TGT GGC AGC CAC CAC CCC TGC ACC AAC GGA GGC   784
Lys Asp Leu Asn Tyr Cys Gly Ser His His Pro Cys Thr Asn Gly Gly
                140                 145                 150
```

FIG. 2A

```
ACG TGC ATC AAC GCC GAG CCT GAC CAG TAC CGC TGC ACC TGC CCT GAC  832
Thr Cys Ile Asn Ala Glu Pro Asp Gln Tyr Arg Cys Thr Cys Pro Asp
            155                 160                 165
GGC TAC TCG GGC AGG AAC TGT GAG AAG GCT GAG CAC GCC TGC ACC TCC  880
Gly Tyr Ser Gly Arg Asn Cys Glu Lys Ala Glu His Ala Cys Thr Ser
        170                 175                 180
AAC CCG TGT GCC AAC GGG GGC TCT TGC CAT GAG GTG CCG TCC GGC TTC  928
Asn Pro Cys Ala Asn Gly Gly Ser Cys His Glu Val Pro Ser Gly Phe
    185                 190                 195
GAA TGC CAC TGC CCA TCG GGC TGG AGC GGG CCC ACC TGT GCC CTT GAC  976
Glu Cys His Cys Pro Ser Gly Trp Ser Gly Pro Thr Cys Ala Leu Asp
200                 205                 210                 215
ATC GAT GAG TGT GCT TCG AAC CCG TGT GCG GCC GGT GGC ACC TGT GTG 1024
Ile Asp Glu Cys Ala Ser Asn Pro Cys Ala Ala Gly Gly Thr Cys Val
                220                 225                 230
GAC CAG GTG GAC GGC TTT GAG TGC ATC TGC CCC GAG CAG TGG GTG GGG 1072
Asp Gln Val Asp Gly Phe Glu Cys Ile Cys Pro Glu Gln Trp Val Gly
            235                 240                 245
GCC ACC TGC CAG CTG GAC GCC AAT GAG TGT GAA GGG AAG CCA TGC CTT 1120
Ala Thr Cys Gln Leu Asp Ala Asn Glu Cys Glu Gly Lys Pro Cys Leu
        250                 255                 260
AAC GCT TTT TCT TGC AAA AAC CTG ATT GGC GGC TAT TAC TGT GAT TGC 1168
Asn Ala Phe Ser Cys Lys Asn Leu Ile Gly Gly Tyr Tyr Cys Asp Cys
    265                 270                 275
ATC CCG GGC TGG AAG GGC ATC AAC TGC CAT ATC AAC GTC AAC GAC TGT 1216
Ile Pro Gly Trp Lys Gly Ile Asn Cys His Ile Asn Val Asn Asp Cys
280                 285                 290                 295
CGC GGG CAG TGT CAG CAT GGG GGC ACC TGC AAG GAC CTG GTG AAC GGG 1264
Arg Gly Gln Cys Gln His Gly Gly Thr Cys Lys Asp Leu Val Asn Gly
                300                 305                 310
TAC CAG TGT GTG TGC CCA CGG GGC TTC GGA GGC CGG CAT TGC GAG CTG 1312
Tyr Gln Cys Val Cys Pro Arg Gly Phe Gly Gly Arg His Cys Glu Leu
            315                 320                 325
GAA CGA GAC AAG TGT GCC AGC AGC CCC TGC CAC AGC GGC GGC CTC TGC 1360
Glu Arg Asp Lys Cys Ala Ser Ser Pro Cys His Ser Gly Gly Leu Cys
        330                 335                 340
GAG GAC CTG GCC GAC GGC TTC CAC TGC CAC TGC CCC CAG GGC TTC TCC 1408
Glu Asp Leu Ala Asp Gly Phe His Cys His Cys Pro Gln Gly Phe Ser
    345                 350                 355
```

FIG. 2B

```
GGG CCT CTC TGT GAG GTG GAT GTC GAC CTT TGT GAG CCA AGC CCC TGC 1456
Gly Pro Leu Cys Glu Val Asp Val Asp Leu Cys Glu Pro Ser Pro Cys
360                 365                 370                 375
CGG AAC GGC GCT CGC TGC TAT AAC CTG GAG GGT GAC TAT TAC TGC GCC 1504
Arg Asn Gly Ala Arg Cys Tyr Asn Leu Glu Gly Asp Tyr Tyr Cys Ala
                380                 385                 390
TGC CCT GAT GAC TTT GGT GGC AAG AAC TGC TCC GTG CCC CGC GAG CCG 1552
Cys Pro Asp Asp Phe Gly Gly Lys Asn Cys Ser Val Pro Arg Glu Pro
            395                 400                 405
TGC CCT GGC GGG GCC TGC AGA GTG ATC GAT GGC TGC GGG TCA GAC GCG 1600
Cys Pro Gly Gly Ala Cys Arg Val Ile Asp Gly Cys Gly Ser Asp Ala
        410                 415                 420
GGG CCT GGG ATG CCT GGC ACA GCA GCC TCC GGC GTG TGT GGC CCC CAT 1648
Gly Pro Gly Met Pro Gly Thr Ala Ala Ser Gly Val Cys Gly Pro His
    425                 430                 435
GGA CGC TGC GTC AGC CAG CCA GGG GGC AAC TTT TCC TGC ATC TGT GAC 1696
Gly Arg Cys Val Ser Gln Pro Gly Gly Asn Phe Ser Cys Ile Cys Asp
440                 445                 450                 455
AGT GGC TTT ACT GGC ACC TAC TGC CAT GAG AAC ATT GAC GAC TGC CTG 1744
Ser Gly Phe Thr Gly Thr Tyr Cys His Glu Asn Ile Asp Asp Cys Leu
                460                 465                 470
GGC CAG CCC TGC CGC AAT GGG GGC ACA TGC ATC GAT GAG GTG GAC GCC  1792
Gly Gln Pro Cys Arg Asn Gly Gly Thr Cys Ile Asp Glu Val Asp Ala
            475                 480                 485
TTC CGC TGC TTC TGC CCC AGC GGT TGG GAG GGC GAG CTC TGC GAC ACC  1840
Phe Arg Cys Phe Cys Pro Ser Gly Trp Glu Gly Glu Leu Cys Asp Thr
        490                 495                 500
AAT CCC AAC GAC TGC CTT CCC GAT CCC TGC CAC AGC CGC GGC CGC TGC  1888
Asn Pro Asn Asp Cys Leu Pro Asp Pro Cys His Ser Arg Gly Arg Cys
    505                 510                 515
TAC GAC CTG GTC AAT GAC TTC TAC TGT GCG TGC GAC GAC GGC TGG AAG  1936
Tyr Asp Leu Val Asn Asp Phe Tyr Cys Ala Cys Asp Asp Gly Trp Lys
520                 525                 530                 535
GGC AAG ACC TGC CAC TCA CGC GAG TTC CAG TGC GAT GCC TAC ACC TGC  1984
Gly Lys Thr Cys His Ser Arg Glu Phe Gln Cys Asp Ala Tyr Thr Cys
                540                 545                 550
AGC AAC GGT GGC ACC TGC TAC GAC AGC GGC GAC ACC TTC CGC TGC GCC  2032
Ser Asn Gly Gly Thr Cys Tyr Asp Ser Gly Asp Thr Phe Arg Cys Ala
            555                 560                 565
TGC CCC CCC GGC TGG AAG GGC AGC ACC TGC GCC GTC GCC AAG AAC AGC  2080
Cys Pro Pro Gly Trp Lys Gly Ser Thr Cys Ala Val Ala Lys Asn Ser
        570                 575                 580
```

FIG. 2C

```
AGC TGC CTG CCC AAC CCC TGT GTG AAT GGT GGC ACC TGC GTG GGC AGC   2128
Ser Cys Leu Pro Asn Pro Cys Val Asn Gly Gly Thr Cys Val Gly Ser
    585                 590                 595
GGG GCC TCC TTC TCC TGC ATC TGC CGG GAC GGC TGG GAG GGT CGT ACT   2176
Gly Ala Ser Phe Ser Cys Ile Cys Arg Asp Gly Trp Glu Gly Arg Thr
600                 605                 610                 615
TGC ACT CAC AAT ACC AAC GAC TGC AAC CCT CTG CCT TGC TAC AAT GGT   2224
Cys Thr His Asn Thr Asn Asp Cys Asn Pro Leu Pro Cys Tyr Asn Gly
                620                 625                 630
GGC ATC TGT GTT GAC GGC GTC AAC TGG TTC CGC TGC GAG TGT GCA CCT   2272
Gly Ile Cys Val Asp Gly Val Asn Trp Phe Arg Cys Glu Cys Ala Pro
            635                 640                 645
GGC TTC GCG GGG CCT GAC TGC CGC ATC AAC ATC GAC GAG TGC CAG TCC   2320
Gly Phe Ala Gly Pro Asp Cys Arg Ile Asn Ile Asp Glu Cys Gln Ser
        650                 655                 660
TCG CCC TGT GCC TAC GGG GCC ACG TGT GTG GAT GAG ATC AAC GGG TAT   2368
Ser Pro Cys Ala Tyr Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr
    665                 670                 675
CGC TGT AGC TGC CCA CCC GGC CGA GCC GGC CCC CGG TGC CAG GAA GTG   2416
Arg Cys Ser Cys Pro Pro Gly Arg Ala Gly Pro Arg Cys Gln Glu Val
680                 685                 690                 695
ATC GGG TTC GGG AGA TCC TGC TGG TCC CGG GGC ACT CCG TTC CCA CAC   2464
Ile Gly Phe Gly Arg Ser Cys Trp Ser Arg Gly Thr Pro Phe Pro His
                700                 705                 710
GGA AGC TCC TGG GTG GAA GAC TGC AAC AGC TGC CGC TGC CTG GAT GGC   2512
Gly Ser Ser Trp Val Glu Asp Cys Asn Ser Cys Arg Cys Leu Asp Gly
            715                 720                 725
CGC CGT GAC TGC AGC AAG GTG TGG TGC GGA TGG AAG CCT TGT CTG CTG   2560
Arg Arg Asp Cys Ser Lys Val Trp Cys Gly Trp Lys Pro Cys Leu Leu
        730                 735                 740
GCC GGC CAG CCC GAG GCC CTG AGC GCC CAG TGC CCA CTG GGG CAA AGG   2608
Ala Gly Gln Pro Glu Ala Leu Ser Ala Gln Cys Pro Leu Gly Gln Arg
    745                 750                 755
TGC CTG GAG AAG GCC CCA GGC CAG TGT CTG CGA CCA CCC TGT GAG GCC   2656
Cys Leu Glu Lys Ala Pro Gly Gln Cys Leu Arg Pro Pro Cys Glu Ala
760                 765                 770                 775
TGG GGG GAG TGC GGC GCA GAA GAG CCA CCG AGC ACC CCC TGC CTG CCA   2704
Trp Gly Glu Cys Gly Ala Glu Glu Pro Pro Ser Thr Pro Cys Leu Pro
                780                 785                 790
CGC TCC GGC CAC CTG GAC AAT AAC TGT GCC CGC CTC ACC TTG CAT TTC   2752
Arg Ser Gly His Leu Asp Asn Asn Cys Ala Arg Leu Thr Leu His Phe
            795                 800                 805
```

FIG. 2D

```
AAC CGT GAC CAC GTG CCC CAG GGC ACC ACG GTG GGC GCC ATT TGC TCC  2800
Asn Arg Asp His Val Pro Gln Gly Thr Thr Val Gly Ala Ile Cys Ser
        810                 815                 820
GGG ATC CGC TCC CTG CCA GCC ACA AGG GCT GTG GCA CGG GAC CGC CTG  2848
Gly Ile Arg Ser Leu Pro Ala Thr Arg Ala Val Ala Arg Asp Arg Leu
    825                 830                 835
CTG GTG TTG CTT TGC GAC CGG GCG TCC TCG GGG GCC AGT GCT GTG GAG  2896
Leu Val Leu Leu Cys Asp Arg Ala Ser Ser Gly Ala Ser Ala Val Glu
840                 845                 850                 855
GTG GCC GTG TCC TTC AGC CCT GCC AGG GAC CTG CCT GAC AGC AGC CTG  2944
Val Ala Val Ser Phe Ser Pro Ala Arg Asp Leu Pro Asp Ser Ser Leu
                860                 865                 870
ATC CAG GGC GCG GCC CAC GCC ATC GTG GCC GCC ATC ACC CAG CGG GGG  2992
Ile Gln Gly Ala Ala His Ala Ile Val Ala Ala Ile Thr Gln Arg Gly
            875                 880                 885
AAC AGC TCA CTG CTC CTG GCT GTC ACC GAG GTC AAG GTG GAG ACG GTT  3040
Asn Ser Ser Leu Leu Leu Ala Val Thr Glu Val Lys Val Glu Thr Val
        890                 895                 900
GTT ACG GGC GGC TCT TCC ACA GGT CTG CTG GTG CCT GTG CTG TGT GGT  3088
Val Thr Gly Gly Ser Ser Thr Gly Leu Leu Val Pro Val Leu Cys Gly
    905                 910                 915
GCC TTC AGC GTG CTG TGG CTG GCG TGC GTG GTC CTG TGC GTG TGG TGG  3136
Ala Phe Ser Val Leu Trp Leu Ala Cys Val Val Leu Cys Val Trp Trp
920                 925                 930                 935
ACA CGC AAG CGC AGG AAA GAG CGG GAG AGG AGC CGG CTG CCG CGG GAG  3184
Thr Arg Lys Arg Arg Lys Glu Arg Glu Arg Ser Arg Leu Pro Arg Glu
                940                 945                 950
GAG AGC GCC AAC AAC CAG TGG GCC CCG CTC AAC CCC ATC CGC AAC CCC  3232
Glu Ser Ala Asn Asn Gln Trp Ala Pro Leu Asn Pro Ile Arg Asn Pro
            955                 960                 965
ATT GAG CGG CCG GGG GGG CAC AAG GAC GTG CTC TAC CAG TGC AAG AAC  3280
Ile Glu Arg Pro Gly Gly His Lys Asp Val Leu Tyr Gln Cys Lys Asn
        970                 975                 980
TTC ACT CCA CCG CCG CGC AGG CGC TGC CCG GGC CGG CCG GCC ACG CGG  3328
Phe Thr Pro Pro Pro Arg Arg Arg Cys Pro Gly Arg Pro Ala Thr Arg
    985                 990                 995
CCG TCA GGG AGG ATG AGG AGG ACG AGG ATC TTG GCC GCG GTG AGG AGG  3376
Pro Ser Gly Arg Met Arg Arg Thr Arg Ile Leu Ala Ala Val Arg Arg
1000                1005                1010                1015
ACT CCC TGG AGG CGG AGA AGT TCC TCT CAC ACA AAT TCA CCA AAG ATC  3424
Thr Pro Trp Arg Arg Arg Ser Ser Ser His Thr Asn Ser Pro Lys Ile
                1020                1025                1030
```

FIG. 2E

```
CTG GCC GCT CGC CGG GGA GGC CGG CCC ACT GGG CCT CAG GCC CCA AAG  3472
Leu Ala Ala Arg Arg Gly Gly Arg Pro Thr Gly Pro Gln Ala Pro Lys
            1035                1040                1045
TGG ACA ACC GCG CGG TCA GGA GCA TCA ATG AGG CCC GCT ACG TCG GCA  3520
Trp Thr Thr Ala Arg Ser Gly Ala Ser Met Arg Pro Ala Thr Ser Ala
        1050                1055                1060
AGG GAA GTA GGG CGG CTG CAG CTG GGC CGG GAC CCA GGG CCC TCG GTG  3568
Arg Glu Val Gly Arg Leu Gln Leu Gly Arg Asp Pro Gly Pro Ser Val
    1065                1070                1075
GGA GCC ATG CCG TCT GCC GGA CCC GGA GGC CGA GGC CAT GTG CAT AGT  3616
Gly Ala Met Pro Ser Ala Gly Pro Gly Gly Arg Gly His Val His Ser
1080                1085                1090                1095
TTC TTT ATT TTG TGT AAA AAA ACC ACC AAA AAC AAA AAC CAA ATG TTT  3664
Phe Phe Ile Leu Cys Lys Lys Thr Thr Lys Asn Lys Asn Gln Met Phe
                1100                1105                1110
ATT TTC TAC GTT TCT TTA ACC TTG TAT AAA TTA TTC AGT AAC TGT CAG  3712
Ile Phe Tyr Val Ser Leu Thr Leu Tyr Lys Leu Phe Ser Asn Cys Gln
            1115                1120                1125
GCT GAA AAC AAT GGA GTA TTC TCG GAT AGT TGC TAT TTT TGT AAA GTA  3760
Ala Glu Asn Asn Gly Val Phe Ser Asp Ser Cys Tyr Phe Cys Lys Val
        1130                1135                1140
GCC GTG CGT GGC ACT CGC TGT ATG AAA GGA GAG AGC AAA GGG TGT CTG  3808
Ala Val Arg Gly Thr Arg Cys Met Lys Gly Glu Ser Lys Gly Cys Leu
    1145                1150                1155
CGT CGT CAC CAA ATC GTC GCG TTT GTT ACC AGA GGT TGT GCA CTG TTT  3856
Arg Arg His Gln Ile Val Ala Phe Val Thr Arg Gly Cys Ala Leu Phe
1160                1165                1170                1175
ACA GAA TCT TCC TTT TAT TCC TCA CTC GGG TTT CTC TGT GCT CCA GGC  3904
Thr Glu Ser Ser Phe Tyr Ser Ser Leu Gly Phe Leu Cys Ala Pro Gly
                1180                1185                1190
CAA AGT GCC GGT GAG ACC CAT GGC TGT GTT GGT GTG GCC CAT GGC TGT  3952
Gln Ser Ala Gly Glu Thr His Gly Cys Val Gly Val Ala His Gly Cys
            1195                1200                1205
TGG TGG GAC CCG TGG CTG ATG GTG TGG CCT GTG GCT GTC GGT GGG ACT  4000
Trp Trp Asp Pro Trp Leu Met Val Trp Pro Val Ala Val Gly Gly Thr
        1210                1215                1220
CGT GGC TGT CAA TGG GAC CTG TGG CTG TCG GTG GGA CCT ACG GTG GTC  4048
Arg Gly Cys Gln Trp Asp Leu Trp Leu Ser Val Gly Pro Thr Val Val
    1225                1230                1235
```

FIG. 2F

GGT GGG ACC CTG GTT ATT GAT GTG GCC CTG GCT GCC GGC ACG GCC CGT 4096
Gly Gly Thr Leu Val Ile Asp Val Ala Leu Ala Ala Gly Thr Ala Arg
1240 1245 1250 1255
GGC TGT TG ACGCACCTGT GGTTGTTAGT GGGGCCTGAG GTCATCGGCG TGGCCCAAGG 4154
Gly Cys
CCGGCAGGTC AACCTCGCGC TTGCTGGCCA GTCCACCCTG CCTGCCGTCT GTGCTTCCTC 4214

CTGCCCAGAA CGCCCGCTCC AGCGATCTCT CCACTGTGCT TTCAGAAGTG CCCTTCCTGC 4274

TGCGCAGTTC TCCCATCCTG GGACGGCGGC AGTATTGAAG CTCGTGACAA GTGCCTTCAC 4334

ACAGACCCCT CGCAACTGTC CACGCGTGCC GTGGCACCAG GCGCTGCCCA CCTGCCGGCC 4394

CCGGCCGCCC CTCCTCGTGA AAGTGCATTT TTGTAAATGT GTACATATTA AAGGAAGCAC 4454

TCTGTATAAA AAAAAAAAAC CGGAATTCC 4483

FIG. 2G

CAGGTGGCGTCAGCATCGGGACAGTTCGAGCTGGAGATCTTATCCGTGCAGAATGTGAACGGCGTGCT
GCAGAACGGGAACTGCTGCGACGGCACTCGAAACCCCGGAGATAAAAAGTGCACCAGAGATGAGTGTG
ACACCTACTTTAAAGTTTGCCTGAAGGAGTACCAGTCGCGGGTCACTGCTGGCGGCCCTTGCAGCTTC
GGATCCAAATCCACCCCTGTCATCGGCGGGAATACCTTCAATTTAAAGTACAGCCGGAATAATGAAAA
GAACCGGATTGTTATCCCTTTCACGTTCGCCTGGCCGAGATCCTACACGTTGCTTGTTGAGGCATGGG
ATTACAATGATAACTCTACTAATCCCGATCGCATAATTGAGAAGGCATCCCACTCTGGCATGATCAAT
CCAAGCCGTCAGTGGCAGACGTTGAAACATAACACAGGAGCTGCCCACTTTGAGTATCAAATCCGTGT
GACTTGCGCAGAACATTACTATGGCTTTGGATGCAACAAGTTTTGTCGACCGAGAGATGACTTCTTCA
CTCACCATACCTGTGACCAGAATGGCAACAAAACCTGCTTGGAAGGCTGGACGGGACCAGAATGCAAC
AAAGCTATTTGTCGTCAGGGATGTAGCCCCAAGCATGGTTCTTGCACAGTTCCAGGAGAGTGCAGGTG
TCAGTATGGATGGCAAGGCCAGTACTGTGATAAGTGCATTCCACACCCGGGATGTGTCCATGGCACTT
GCATTGAACCATGGCAGTGCCTCTGTGAAACCAACTGGGGTGGTCAGCTCTGTGACAAAGACCTGAAC
TACTGTGGAACCCACCCACCCTGTTTGAATGGTGGTACCTGCAGCAACACTGGCCCCGATAAATACCA
GTGTTCCTGCCCTGAGGGTTACTCAGGACAGAACTGTGAAATAGCGGAGCATGCGTGCCTCTCTGATC
CGTGCCACAACGGAGGAAGCTGCCTAGAAACGTCTACAGGATTTGAATGTGTGTGTGCACCTGGCTGG
GCTGGACCAACTTGCACTGATAATATTGATGATTGTTCTCCAAATCCCTGTGGTCATGGAGGAACTTG
CCAAGATCTAGTTGATGGATTTAAGTGTATTTGCCCACCTCAGTGGACTGGCAAAACATGCCAGCTAG
ATGCGAATGAATGTGAGGGCAAACCCTGTGTCAATGCCAACTCCTGCAGGAACTTGATTGGCAGCTAC
TATTGTGACTGCATTACTGGCTGGTCTGGCCACAACTGTGATATAAATATTAATGATTGTCGTGGACA
ATGTCAGAATGGAGGATCCTGTCGGGACTTGGTTAATGGTTATCGGTGCATCTGTTCACCTGGCTATG
CAGGAGATCACTGTGAGAAAGACATCAATGAATGTGCAAGTAACCCTTGCATGAATGGGGGTCACTGC
CAGGATGAAATCAATGGATTCCAATGTCTGTGTCCTGCTGGTTTCTCAGGAAACCTCTGTCAGCTGGA
TATAGACTACTGTGAGCCAAACCCTTGCCAGAACGGTGCCCAGTGCTTCAATCTTGCTATGGACTATT
TCTGTAACTGCCCTGAAGATTACGAAGGCAAGAACTGCTCCCACCTGAAAGATCACTGCCGCACAACT
CCTTGTGAAGTAATCGACAGCTGTACAGTGGCAGTGGCTTCTAACAGCACACCAGAAGGAGTTCGTTA
CATTTCTTCAAATGTCTGTGGTCCTCATGGAAAATGCAAGAGCCAAGCAGGTGGAAAATTCACCTGTG
AATGCAACAAAGGATTCACTGGCACCTACTGTCATGAGAATATCAATGACTGTGAGAGCAACCCCTGT
AAAAATGGTGGCACTTGTATTGACGGTGTAAACTCCTACAAATGTATTTGTAGTGATGGATGGGAAGG
AACATATTGTGAAACAAATATTAATGACTGCAGTAAAAACCCCTGCCACAATGGAGGAACTTGCCGAG
ACTTGGTCAATGACTTCTTCTGTGAATGTAAAAATGGGTGGAAAGGAAAAACTTGCCACTCTCGTGAC
AGCCAGTGTGATGAGGCAACATGCAATAATGGAGGAACATGTTATGATGAGGGGGACACTTTCAAGTG
CATGTGTCCTGCAGGATGGGAAGGAGCCACTTGTAATATAGCAAGGAACAGCAGCTGCCTGCCAAACC
CCTGTCACAATGGTGGTACCTGTGTAGTTAGTGGGGATTCTTTCACTTGTGTCTGCAAGGAGGGCTGG
GAAGGACCGACATGTACTCAGAACACAAATGACTGCAGTCCTCATCCTTGTTACAACAGTGGTACTTG
TGTGGATGGAGACAACTGGTACCGCTGTGAGTGCGCTCCCGGCTTCGCAGGTCCCGACTGTAGGATCA
ACATCAATGAATGTCAGTCTTCACCCTGTGCCTTTGGGGCTACTTGTGTGGATGAAATTAATGGGTAC
CGTTGCATTTGTCCACCGGGTCGCAGTGGTCCAGGATGCCAGGAAGTTACAGGGAGGCCTTGCTTTAC
CAGTATTCGAGTAATGCCAGACGGTGCTAAGTGGGATGATGACTGTAATACTTGTCAGTGTTTGAATG
GAAAAGTCACCTGTTCTAAGGTTTGGTGTGGTCCTCGACCTTGTATAATACATGCCAAAGGTCATAAT
GAATGCCCAGCTGGACACGCTTGTGTTCCTGTTAAAGAAGACCATTGTTTCACTCATCCTTGTGCTGC

FIG. 3A

AGTGGGTGAATGCTGGCCTTCTAATCAGCAGCCTGTGAAGACCAAATGCAATTCTGATTCTTATTACC
AAGATAATTGTGCCAACATCACCTTCACCTTTAATAAGGAAATGATGGCACCAGGCCTTACCACGGAG
CACATTTGCAGTGAATTGAGGAATCTGAATATCCTGAAGAATGTTTCTGCTGAATATTCCATCTATAT
TACCTGTGAGCCTTCACACTTGGCAAATAATGAAATACATGTTGCTATTTCTGCTGAAGATATAGGAG
AAGATGAAAACCCAATCAAGGAAATCACAGATAAGATTATTGACCTTGTCAGTAAGCGTGATGGAAAC
AACACACTAATTGCTGCAGTCGCAGAAGTCAGAGTACAAAGGCGACCAGTTAAGAACAAAACAGATTT
CTTGGTGCCATTACTGAGCTCAGTCTTAACAGTAGCCTGGATCTGCTGTCTGGTAACTGTTTTCTATT
GGTGCATTCAAAAGCGCAGAAAGCAGAGCAGCCATACTCACACAGCATCTGATGACAACACCACCAAC
AACGTAAGGGAGCAGCTGAATCAGATTAAAAACCCCATAGAGAAACACGGAGCAAATACTGTTCCAAT
TAAAGACTATGAAAACAAAAACTCTAAAATCGCCAAAATAAGGACGCACAATTCAGAAGTGGAGGAAG
ATGACATGGACAAACACCAGCAAAAGGCCCGGTTTGCCAAGCAGCCAGCGTACACTTTGGTAGACAGA
GATGAAAAGCCACCCAACAGCACACCCACAAAACACCCAAACTGGACAAATAAACAGGACAACAGAGA
CTTGGAAAGTGCACAAAGTTTAAATAGAATGGAGTACATTGTATAG

FIG. 3B

```
QVASASGQFE LEILSVQNVN GVLQNGNCCD GTRNPGDKKC TRDECDTYFK   50
     ^
VCLKEYQSRV TAGGPCSFGS KSTPVIGGNT FNLKYSRNNE KNRIVIPFSF  100
AWPRSYTLLV EAWDYNDNST NPDRIIEKAS HSGMINPSRQ WQTLKHNTGA  150
AHFEYQIRVT CAEHYYGFGC NKFCRPRDDF FTEHTCDQNG NKTCLEGWTG  200
      **********DSL DOMAIN**********
PECNKAICRQ GCSPKHGSCT VPGECRCQYG WQGQYCDKCI PHPGCVHGTC  250
***    <-------------EGF 1------------><-----------
IEPWQCLCET NWGGQLCDKD LNYCGTHPPC LNGGTCSNTG PDKYQCSCPE  300
-----EGF 2--------------><-----------------EGF 3----
GYSGQNCEIA EHACLSDPCH NGGSCLETST GFECVCAPGW AGPTCTDNID  350
------------><-----------------EGF 4---------------
DCSPNPCGHG GTCQDLVDGF KCICPPQWTG KTCQLDANEC EGKPCVNANS  400
><-----------------EFG 5---------------><----------
CRNLIGSYYC DCITGWSGHN CDININDCRG QCQNGGSCRD LVNGYRCICS  450
-------EFG 6---------------><---------------EFG 7---
PGYAGDHCEK DINECASNPC MNGGHCQDEI NGFQCLCPAG FSGNLCQLDI  500
-------------><-----------------EFG 8--------------
DYCEPNPCQN GAQCFNLAMD YFCNCPEDYE GKNCSHLKDH CRTTPCEVID  550
-><-----------------EFG 9----------------><---------
SCTVAVASNS TPEGVRYISS NVCGPHGKCK SQAGGKFTCE CNKGFTGTYC  600
----------------------EFG 10------------------------
HENINDCESN PCKNGGTCID GVNSYKCICS DGWEGTYCET NINDCSKNPC  650
-----><----------------EFG 11----------------><-----
HNGGTCRDLV NDFFCECKNG WKGKTCHSRD SQCDEATCNN GGTCYDEGDT  700
------------EFG 12--------------><-----------------
FKCMCPAGWE GATCNIARNS SCLPNPCHNG GTCVVSGDSF TCVCKEGWEG  750
EGF 13----------------><---------------EGF 14-------
PTCTQNTNDC SPHPCYNSGT CVDGDNWYRC ECAPGFAGPD CRININECQS  800
--------><-----------------EGF 15---------------><--
SPCAFGATCV DEINGYRCIC PPGRSGPGCQ EVTGRPCFTS IRVMPDGAKW  850
---------------EGF 16---------------->
DDDCNTCQCL NGKVTCSKVW CGPRPCIIHA KGHNECPAGH ACVPVKEDHC  900
   <-                    CYSTEINE-RICH REGION
FTHPCAAVGE CWPSNQQPVK TKCNSDSYYQ DNCANITFTF NKEMMAPGLT  950
         ->
TEHICSELRN LNILKNVSAE YSIYITCEPS HLANNEIHVA ISAEDIGEDE 1000
```

FIG. 4A

NPIKEITDKI IDLVSKRDGN NTLIAAVAEV RVQRRPVKNK TDFLVPLLSS 1050

VLTVAWICCL VTVFYWCIQK RRKQSSHTHT ASDDNTTNNV REQLNQIKNP 1100

IEKHGANTVP IKDYENKNSK IAKIRTHNSE VEEDDMDKHQ QKARFAKQPA 1150

YTLVDRDEKP PNSTPTKHPN WTNKQDNRDL ESAQSLNRME YIV 1193

FIG. 4B

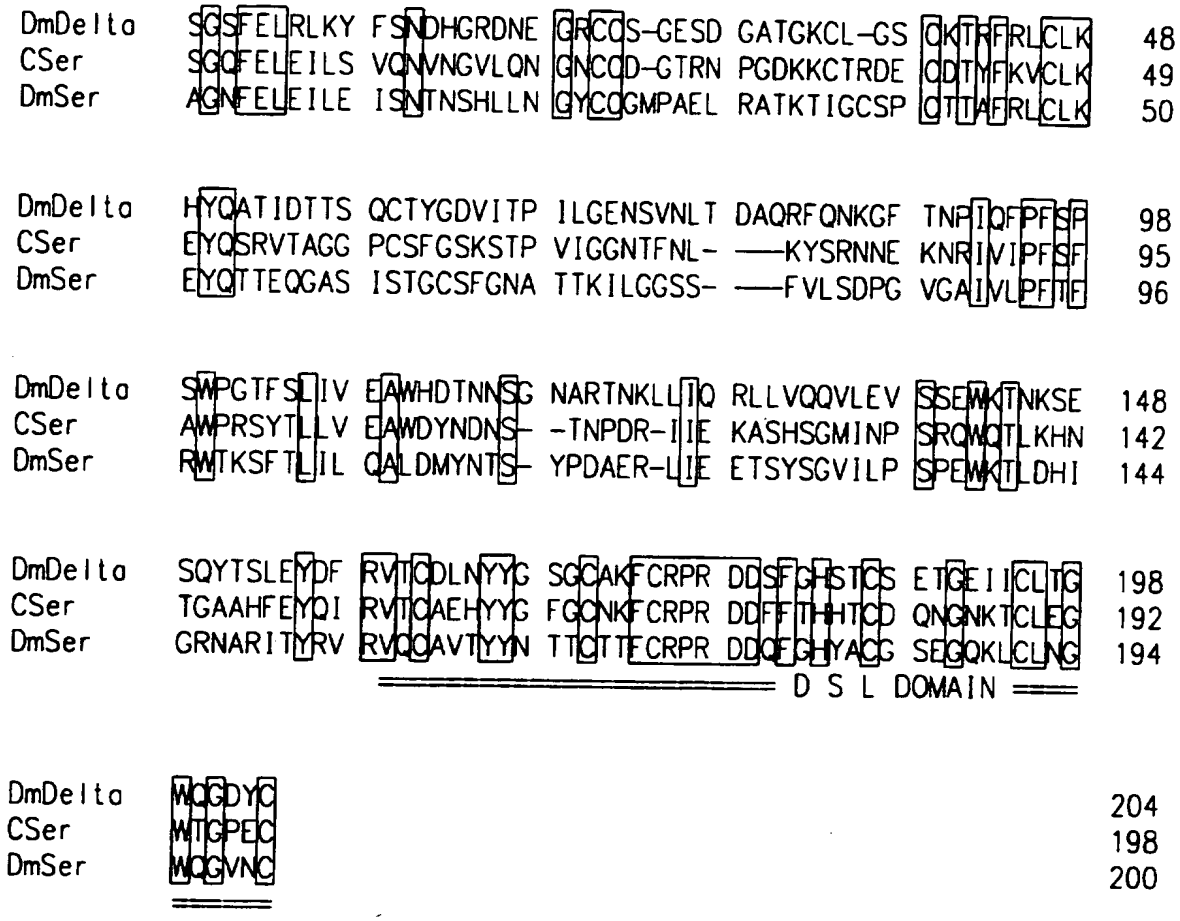

FIG.5

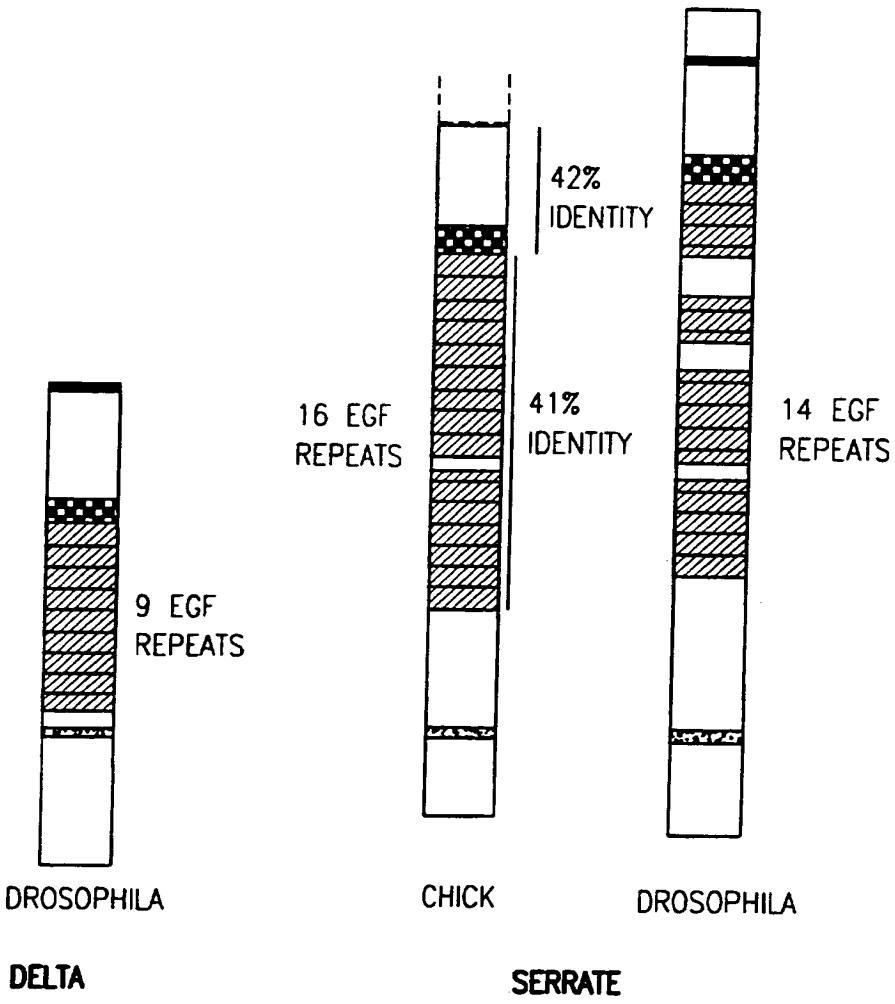

FIG.6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03172

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K14/00; A01N 37/18, 43/04; C07H 17/00; C12N 5/00; C12P 21/06
US CL :530/350, 387.1; 514/2, 44/536/23.1/ 435/69.1, 240.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 387.1; 514/2, 44/536/23.1/ 435/69.1, 240.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	GRAY et al. Human ligands of the notch receptor. Society for Neuroscience Abstraces, 11 November 1995, Vol. 21, No. 1-3, page 1524, abstract 601.1. See entire abstract.	1-82
P, A	CHITNIS et al. Primary neurogenesis in Xenopus embryos regulated by a homologue of the Drosophila neurogenic gene Delta. Nature, 29 June 1995, Vol. 375, pages 761-766. See entire document.	1-82
P, A	HENRIQUE et al. Expression of a Delta homologue in prospective neurons in the chick. Nauture, 29 June 1995, Vol. 375, pages 787-790. See entire document.	1-82

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
08 JULY 1996	30 JUL 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Karen Cochrane Carlson, Ph.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03172

Box I Observations where certain claims were found unsearchable (Continuation f item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3 ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3 ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03172

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	LINDSELL et al. Jagged: A mammalian ligand that activates Notch1. Cell. 24 March 1995, Vol. 80, pages 909-917. See entire document.	1-82

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03172

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)s 1-29, 34-68, 78, and 79, drawn to DNA and amino acid seqeunces encoding Serrate protein.

Group II, claim(s) 30-33, drawn to an antibody against Serrate protein.

Group III, claim(s) 69-73 and 77, drawn to a method of treating disease with Serrate protein.

Group IV, claim(s) 74 and 75, drawn to a method of treating disease with DNA encoding Serrate protein - gene therapy.

Group V, claim(s) 76, drawn to a method of treating a disease with the antibody against Serrate protein.

Group VI, claim(s) 80, drawn to a method of inhibiting expression of Serrate protein using antisense DNA.

Group VII, claim(s)81, drawn to method for diagnosing a disease via notch:Serrate protein binding assay.

Group VIII, claim(s) 82, drawn to a method for diagnosing a disease via measuring Serrate protein levels.

The inventions listed as Groups I through VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features. for the following reasons:

Groups I and Group II are structurally different compounds/compositions having unique properties not shared by the other. A reference anticipating or rendering obvious the compound/composition of Group I would not necessarily anticipate or make obvious the compound/composition of Group II. Groups III-VIII are properly grouped separately from the main invention of Group I, pursuant to 37 CFR 1.475(d). Therefore, the groupings lack the same or corresponding special technical feature.